MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXIX SEPTEMBER–OCTOBER, 1937 No. 5

PRODUCTION OF CONIDIA BY CORTICIUM INCRUSTANS [1]

MILDRED K. NOBLES [2]

(WITH 26 FIGURES)

This species, under the name *Corticium roseopallens* Burt, was studied by Lyman (**1907**) who described the characteristics displayed by the fungus in culture, with special emphasis on the production of conidia. When in the course of a study of conidia-bearing species of Thelephoraceae, cultures of this species were obtained, it seemed desirable to repeat his observations and extend them where possible.

Rogers (**1935**) found that the American *Corticium roseopallens* Burt was identical with the European *Corticium incrustans* v. Höhn. & Litsch. Since the latter name has priority, it is adopted here.

The fruit body from which the cultures were obtained was collected by Dr. R. F. Cain on a log of *Populus* sp. near Hatchley, Ontario, on October 27, 1934 (University of Toronto Herbarium No. *6549*).

[1] Contribution from the Department of Botany, University of Toronto, Toronto, Ontario.

[2] The author wishes to acknowledge her indebtedness to Professor H. S. Jackson, under whose direction the work was carried on, and to Dr. R. F. Cain, who collected the fruit body from which the cultures were obtained.

[MYCOLOGIA for July–August (**29**: 377–556) was issued August 2, 1937]

CULTURAL STUDIES

Haploid mycelium. The basidiospores, which are hyaline allantoid bodies ranging from 4–5 × 1.5–2.5 μ in size, germinate readily when sown on malt extract agar, the sporelings being large enough to transfer to stock culture tubes in forty-eight hours. According to Lyman (**1907**) the basidiospores retain their viabil-

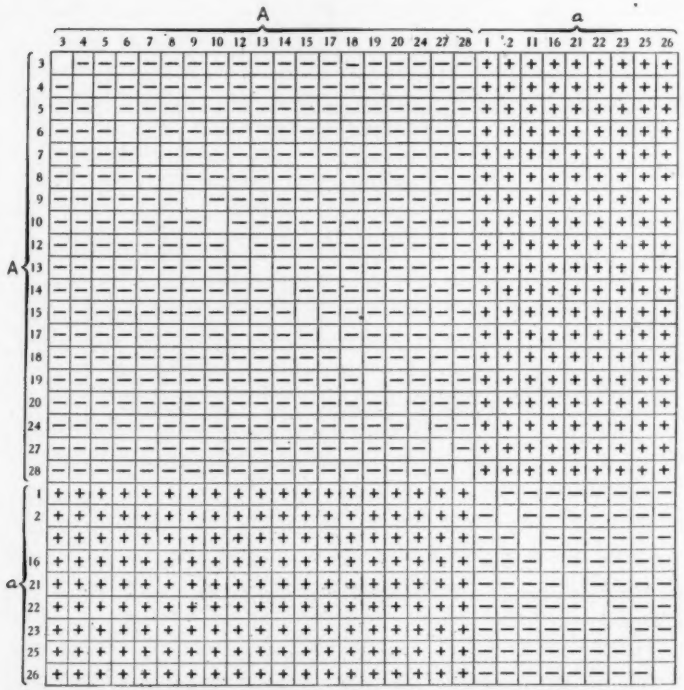


FIG. 1. *Corticium incrustans.* Results obtained by pairing in all possible combinations twenty-eight mycelia, each derived from the germination of a single basidiospore.

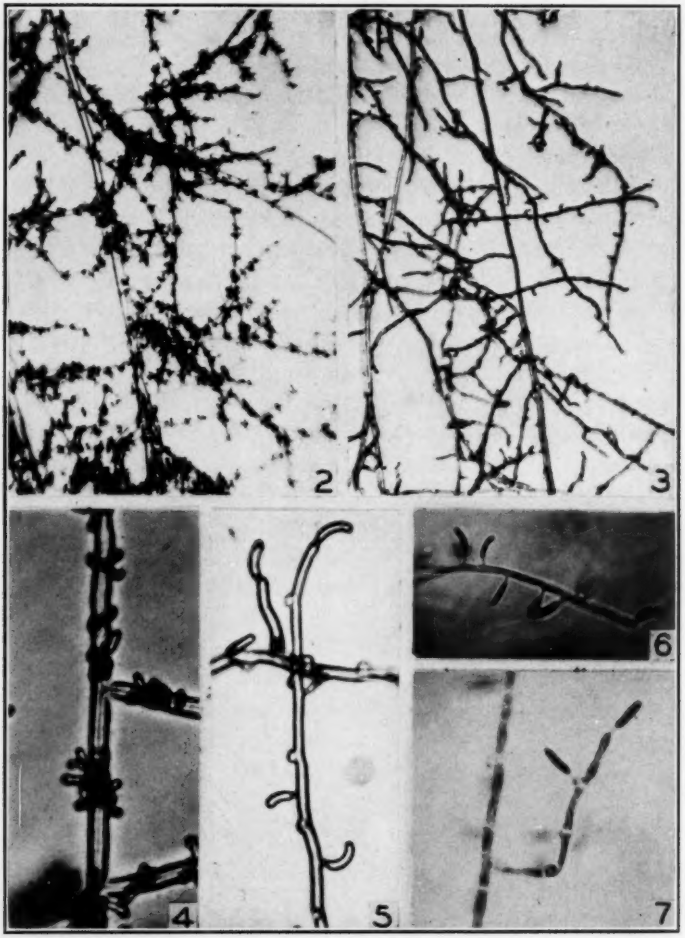
ity for a considerable length of time, up to six months under laboratory conditions, but this has not been verified in the present work.

When grown on malt extract agar the mycelium arising from a single basidiospore is slow-growing, with most of the hyphae sub-

merged or appressed so that the colony appears rather soggy or damp. After some days a slight growth of aerial hyphae develops, which becomes denser with age. Usually the mycelium is white, although in some cultures it has a pale pink color. Microscopic examination shows that the hyphae have simple septa. Lyman (**1907,** *pl. 20, fig. 61*) stated that the basidiospores were irregular in their behavior, germinating to produce mycelium bearing clamp connections in some cases, lacking them in others. This led Martens and Vandendries (**1933**) to conclude that the fungus is homothallic, a conclusion which has not been corroborated in the present work, where clamp connections were never observed on sporelings arising from basidiospores. Furthermore, of the thirty single basidiospores isolated, none has developed clamp connections in the two years since their isolation. Hence it must be concluded that monosporous mycelia of *Corticium incrustans* are haploid.

Conidia. About five days after the germination of a basidiospore, the colony begins to produce conidia (FIG. 2, 4, 6). They resemble the basidiospores in that they are allantoid and hyaline, but they exceed the basidiospores slightly in size, ranging from 5.0–8.9 μ in length by 1.9–3.0 μ in width, averaging 7.1×2.3 μ. Their production is confined to aerial hyphae. The formation of a conidium is initiated by the appearance of a papilla on a hypha, the papilla elongating to a slender sterigma, the end of which increases in size until the characteristic allantoid conidium is produced. On the haploid mycelium a cell may produce one to many conidia, which appear in clumps on small elevations or in whorls. In the moist chambers devised by Vernon (**1931**) the hyphae which grow into the air, bristle with conidia (FIG. 2). Older cultures on Petri dishes have a similar appearance when viewed under a lens, due to the production of conidia on all the aerial hyphae. These conidia give rise to haploid mycelia indistinguishable from those of basidiospore origin.

Pairings of single basidiospore cultures. Two months after their isolation, twenty-eight of the single basidiospore cultures were grown together in pairs in all possible combinations, and the resulting mycelia examined for the presence of clamp connections. The data so obtained are tabulated in figure 1, in which a plus



FIGS. 2–7. Production of conidia. *2*, haploid mycelium and conidia, grown in Vernon cell, × 150 approximately; *3*, diploid mycelium and conidia, × 200 approximately; *4*, haploid mycelium and conidia, × 600 approximately; *5*, diploid mycelium and conidia, × 600 approximately; *6*, conidia on haploid hypha, × 500 approximately; *7*, conidia on diploid hypha, × 500 approximately.

sign indicates the presence of clamp connections, a minus sign their absence. It may be observed that the mycelia fall into two groups, a member of group *A* reacting with a member of group *a* in such a way as to produce a clamp-bearing mycelium, but failing to respond in such a manner to a member of the same group. Hence *Corticium incrustans* is heterothallic and of the bipolar type of interfertility.

Diploid mycelium. The character of the growth of the diploid mycelium obtained by pairing two suitable haploid mycelia, resembles that of the parent haploid mycelia in general, but differs in being more vigorous both in rate of growth and production of aerial hyphae. Microscopically the diploid mycelium differs in the presence of a clamp connection at every septum, and in the less abundant production of conidia. The conidia borne on the diploid mycelium are similar to those of haploid origin in general appearance, but exceed them in size, being 7.6–13.2 × 2.5–3.8 μ, averaging 10.8 × 3.1 μ. These conidia are produced in the same way as the haploid conidia, but, except in rare instances, there is only one conidium on each cell (FIG. 3, 5, 7). Quite frequently a conidium is borne directly on a clamp connection (FIG. 7). The spores may be close together along a hypha but careful observation shows the presence of only one on each cell. Following conidium formation the cell becomes vacuolate, and seemingly never regains sufficient vigor to produce a second spore. In general, conidia which were developed on a clamp-bearing mycelium germinate to give sporelings showing clamp connections immediately.

CYTOLOGICAL STUDIES

A cytological study has been made of haploid and diploid mycelium, conidia, and sporelings. Although the small size of nuclei in rapidly growing hyphae caused considerable difficulty in observations, it is possible to make definite statements about most of the points of interest.

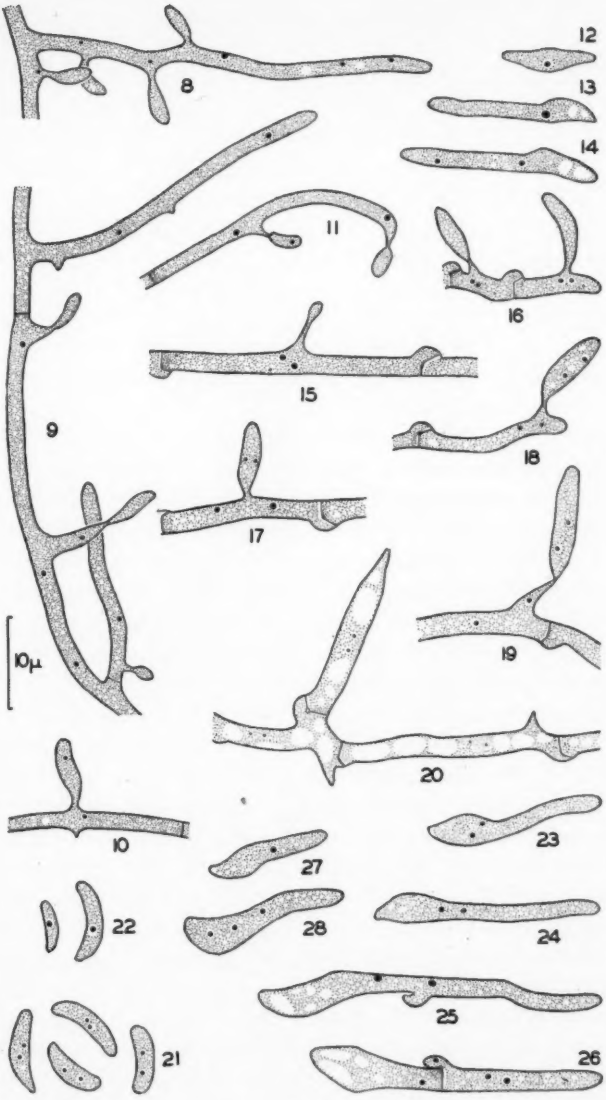
Haploid mycelium. In a haploid mycelium, those cells which are at some distance back from an actively growing hyphal tip are uninucleate, while tip cells and those directly behind the tip are usually long and contain from two to several nuclei spaced at in-

tervals along the cell (FIG. 8, 9). Hence in growing mycelium, septum formation does not follow directly on nuclear divisions, nor does it appear to be associated with these divisions.

Conidia. Conidia are formed on hyphae whose contents show an affinity for stain. The sterigma originates at any point on the cell wall, without reference to the nuclei within the cell (FIG. 8, 9), but before the conidium attains full size, a nucleus is seen at the base of the sterigma (FIG. 8, 9). It is inferred that this nucleus divides, since later stages show a nucleus in the spore, and one within the cell below the spore (FIG. 10, 11). That this occurs is substantiated by the observation of whorls of conidia on a cell. The nuclei of these spores probably originate in the successive divisions of one nucleus, one daughter nucleus migrating into each spore, one remaining in the hypha. Stages in the germination of haploid conidia are illustrated in figures 12–14.

Diploid mycelium. The mycelium bearing clamp connections consists of binucleate cells, the nuclear divisions taking place through the agency of the clamp connections. Conidia are produced at any point on the cell wall or on the clamp connection. As in the haploid mycelium a conidium appears first as a small tapering outgrowth, the end of which swells to form the spore (FIG. 15). When the latter has reached a considerable size, the two nuclei are to be seen at the base of the sterigma (FIG. 16). Although division figures have not been observed, it is assumed that these nuclei undergo a division without the intervention of a clamp connection, since later stages show a binucleate conidium continuous with a cell which is still binucleate (FIG. 17, 18). Following the production of conidia, the cells become vacuolate, and the contents gradually lose their affinity for the stain (FIG. 19, 20).

Vandendries (**1934**b) has described a comparable condition in *Pleurotus pinsitus,* in which conidia are produced on dicaryotic mycelium in a manner resembling that in *Corticium incrustans.* In *P. pinsitus* the usual course is for the two nuclei of the parent cell to migrate into the conidium. He writes (p. 208) "Une conidie latérale, placée entre deux anses d'anastomose, reçoit les deux noyaux de l'article mycélien qui la porte. Ce tronçon limité aux deux extrémités par des cloisons placées aux anses d'anastomose, perd ainsi toute possibilité de vie. On constate dans les



FIGS. 8–26. Cytological studies. 8–11, haploid hyphae and stages in conidium formation; 12–14, stages in germination of haploid conidia; 15–19, stages in formation of conidia by binucleate cells; 20, diploid cells following formation of conidia; 21, binucleate conidia produced on diploid mycelium; 22, uninucleate conidia produced on diploid mycelium; 23–26, stages in germination of binucleate conidia; 27, 28, germination of conidia containing irregular members of nuclei. All × 1400.

préparations des filaments ainsi énucléés et porteurs, entre deux boucles, d'une conidie binucléée." Occasionally Vandendries observed that the nuclei divided, and one pair entered the spore, the cell remaining binucleate. In either case, the formation of a conidium apparently leaves the cell in a weakened condition from which it never recovers, and in this respect *Pleurotus pinsitus* is similar to *Corticium incrustans.*

In describing the formation of conidia on diploid mycelium of *Corticium incrustans,* it was stated that each conidium receives two nuclei (FIG. 21). In general such is the case. Occasionally, however, conidia containing irregular numbers of nuclei were observed in stained preparations (FIG. 22). Of 131 stained conidia counted, 110 or 83.9 per cent were binucleate; 17 or 12.9 per cent were uninucleate, while 3 contained three nuclei and 1 contained four nuclei. Examination of stained preparations has not shown how these one-, three- and four-nucleate spores have formed. It is conceivable, however, that a conidium produced on a clamp connection might receive only the nucleus of the clamp-cell, which had failed to migrate into the penultimate cell following a division. Since many conidia are observed on clamps, this might explain the occurrence of the uninucleate spores. Formation of a spore on a clamp connection might also result in the production of a spore containing three nuclei, if, following nuclear division, the wall cutting off the clamp connection failed to form, and the two nuclei of the end cell, along with the nucleus of the clamp connection, migrated into the conidium. Finally the four-nucleate conidium might arise through the failure of a clamp connection to function in a nuclear division, or by migration of both daughter nuclei of a division preceding conidium formation. Unfortunately these are only suggestions without observations to substantiate them.

In the germination of conidia from a dicaryotic mycelium, the two nuclei of the spore migrate into the germ tube (FIG. 23–26) where the first division is accomplished through the agency of a clamp connection, and thereafter the mycelium is composed of binucleate cells, and indistinguishable from that resulting from the pairing of two compatible haploid mycelia. The few uninucleate conidia produced on the diploid mycelium germinate tardily to

form mycelia like those arising from single basidiospores. In *Pleurotus pinsitus,* Vandendries (**1934**b) found occasional uninucleate conidia on diploid mycelium, but never observed the germination of these. Kaufert (**1935**) described the production of spores in coremia on haploid and diploid cultures of *Pleurotus corticatus.* Of the spores formed on the diploid mycelium, the majority contained two nuclei, but a few contained only a single nucleus. It would appear that such uninucleate spores are exceptional and of slight importance in the life of the fungus.

DISCUSSION

Corticium incrustans is the first species of the Thelephoraceae in which the existence of both haploid and diploid conidia has been established. That these were produced was indicated by Lyman's (**1907**) figures and descriptions, in which he stated that (p. 176) " the germ tubes (of conidia) may or may not bear clamps." The present study has shown that conidia borne on haploid or diploid mycelium repeat the generation on which they are produced.

For a long time diploid spores have been known to occur in rusts and smuts, but their production by a hymenomycetous species has been reported only rarely. Martens and Vandendries (**1933**) reviewed the literature on the subject and concluded (p. 351) " Il n'existe qu'un très petit nombre d'exemples où l'origine et la valeur diploïde des spores accessoires soient réellement établies." Since that time the production of binucleate conidia has been described in five species: *Pholiota aurivella* by Martens and Vandendries (**1933**); *Trametes cinnabarina* by Vandendries (**1934**a); *Pleurotus pinsitus* by Vandendries (**1934**b); *Polyporus squamosus* by Vandendries (**1934**c); *Polyporus corticatus* by Kaufert (**1935**). The present study has added a sixth species to this list.

SUMMARY

Corticium incrustans v. Hohn. & Litsch. is heterothallic and of the bipolar type of interfertility. Conidia are produced on short sterigmata on vegetative hyphae. In haploid mycelium several uninucleate conidia are borne on each cell. These germinate

readily to give rise to new individuals like the parent. In diploid mycelium, one conidium is borne on each cell. These spores are binucleate and give, on germination, new diploid plants.

DIVISION OF BOTANY,
CENTRAL EXPERIMENTAL FARM,
OTTAWA, ONTARIO

LITERATURE CITED

Kaufert, F. The production of asexual spores by *Pleurotus corticatus*. Mycologia **27**: 333–341. 1935.

Lyman, G. R. Culture studies on polymorphism of Hymenomycetes. Proc. Boston Soc. Nat. Hist. **33**: 125–209. 1907.

Martens, P. & Vandendries, R. Le cycle conidien haploïde et diploïde, chez *Pholiota aurivella*. La Cellule **41**: 337–386. 1933.

Rogers, D. P. Notes on the lower Basidiomycetes. Univ. Iowa Studies Nat. Hist. **17**: 1–43. 1935.

Vandendries, R. Contribution a l'étude de la sexualité dans le genre Trametes. Bull. Soc. Myc. Fr. **50**: 98–110. 1934a.

——. La polarité sexuelle et le régime conidien chez *Pleurotus pinsitus*. Ibid. **50**: 203–212. 1934b.

——. Les polarités sexuelles dans le genre *Pholiota*. Ibid. **50**: 270–277. 1934c.

Vernon, T. R. An improved type of moist chamber for studying fungal growth. Ann. Bot. **45**: 733–737. 1931.

THE CULTURAL CHARACTERISTICS OF FOMES CONNATUS [1]

W. A. CAMPBELL

(WITH 2 FIGURES)

INTRODUCTION

In a study of the cultural characteristics of the species of *Fomes*,[2] the writer tried without success to secure *Fomes connatus* (Weinm.) Gill. in pure culture. As Baxter (2) has noted, sporophore tissue isolations are difficult to obtain because the soft, watery sporophores, even when very young, are contaminated by other fungi and bacteria. It is also difficult to isolate from the soft rot produced by *F. connatus* due to the rather constant association of discoloring organisms with its incipient stages. Many attempts were made over a period of two years to isolate the fungus from fresh sporophores and decayed wood and to germinate the basidiospores. These were unsuccessful because of the presence of contaminating fungi or the failure of the spores to germinate. Finally a sporophore was collected which produced viable spores free from contaminants. The fungus, so obtained, was believed to be *F. connatus*, but because of the number of conflicting organisms previously secured, additional spore cultures were deemed essential before any cultural study of the species could be made.

A knowledge of the cultural characteristics of *F. connatus* became increasingly desirable in connection with decay studies in northern hardwoods, since both *Acer Saccharum* and *A. rubrum*, important constituents of these stands, are common hosts for the fungus. During the fall of 1936 a fungus was isolated from a hard maple decay sample sent to Ross W. Davidson, of the Division of Forest Pathology, from the Kane Experimental Forest,

[1] Research supported in part by Emergency Conservation Work.

[2] Campbell, W. A. The cultural characteristics of the species of *Fomes*. Unpublished thesis. Pennsylvania State College 1935.

Pennsylvania, and was identified as *F. connatus* on the basis of a sporophore which it produced in culture. Field records showed that the tree from which the decay sample was obtained had *F. connatus* fruiting on it. Later, Davidson germinated spores obtained from a sporophore collected on *A. Negundo* by Campbell and Overholts at Haun's Bridge, Pennsylvania. These two isolations, whose Forest Pathology reference collection numbers are *71522* and *71339* respectively, proved identical in culture and agreed with the spore isolation obtained several years previously. The cultural characteristics of *F. connatus* as here given are based on these isolations.

FOMES CONNATUS IN CULTURE

Petri dish cultures:[3] Growth very slow forming a mat about 1–2 cm. in diameter in 7 days. Mat white, cottony over inoculum, nearly colorless, thin, short cottony to fine woolly on agar proper; margin colorless to faintly white, thin, even.

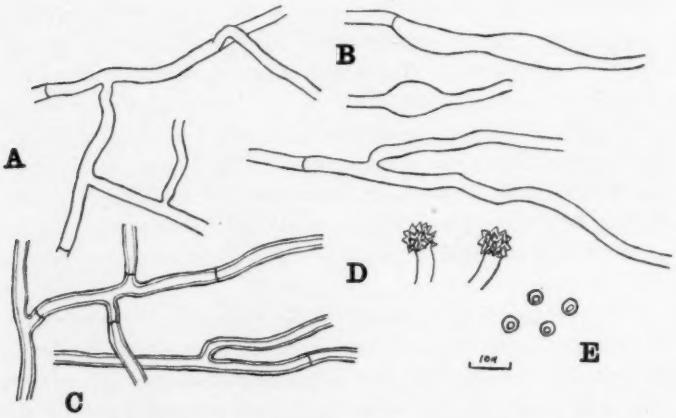


FIG. 1. *A*, submerged hyphae; *B*, swollen cells; *C*, fibrous hyphae from old cultures; *D*, incrusted cystidia from sporophore produced in culture; *E*, basidiospores produced in culture.

[3] All descriptions based on mats grown on 1.5 per cent Difco malt with 2 per cent agar, in diffused light at room temperature, inoculum 2 mm. square.

In 14 days forming a mat 2–3 cm. in diameter (FIG. 2, *A*). Mat usually raised cottony over inoculum becoming more appressed cottony toward margin, very fragile, azonate, occasionally with prominent sectors.

Submerged hyphae 2–5 μ in diameter, very thin-walled, often much contorted and twisted, with abundant cross-walls but no

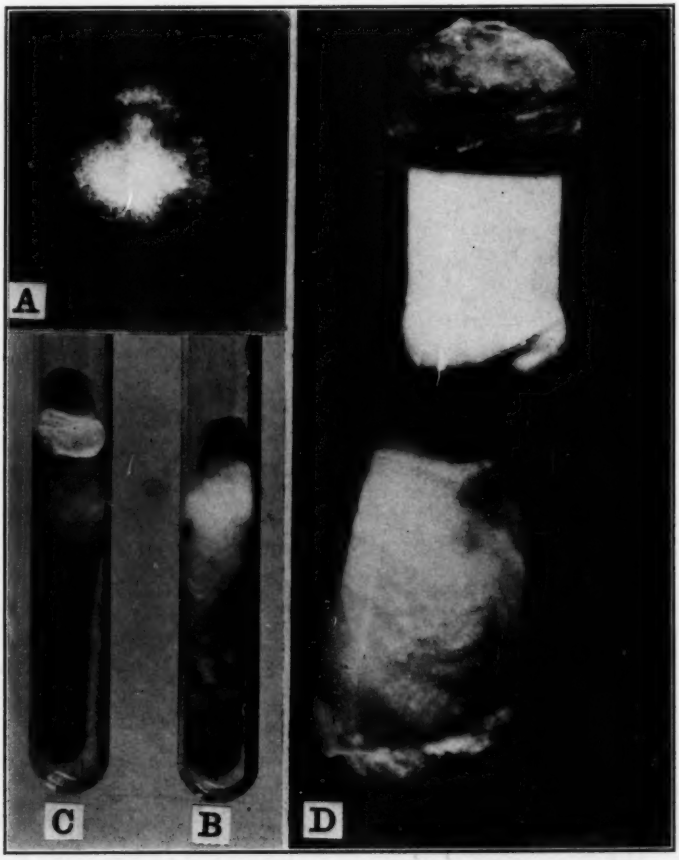


FIG. 2. *A*, mat age 14 days in Petri dish; *B*, age 28 days; *C*, isolation 71522 fruiting in tube, age 6 weeks; *D*, flask culture on maple block, age 2 months. Photographs by M. L. F. Foubert.

clamps (FIG. 1, *A*), swollen cells either terminal or intercalary, common (FIG. 1, *B*); superficial hyphae either same as submerged, thin-walled, staining readily with eosin 2–5 μ or empty, non-staining; fibrous hyphae 2–4 μ non-staining, smooth, with moderately thick walls, rare in young cultures but common in old Petri dish and test tube cultures (FIG. 1, *C*).

Bavendamm's (1) oxidase reaction test[4] gave a faint to moderately strong brown diffusion zone on gallic acid medium with no growth in 7 days; on tannic acid medium a faint diffusion zone with no growth.

Temperature relations: Optimum temperature for growth about 25° C. Average diameters of mats in 7 days, in dark, at constant temperatures as follows: 1.5 cm., 20°; 2.4 cm., 25°; 1.4 cm., 30°; no growth, 35°.

Test-tube cultures: In 14 days forming a raised, white, silky-cottony mound on agar slant with appressed, colorless, cottony marginal region on agar cylinder.

In 28 days with a pronounced white, raised, compacted cottony pad on slant and with an indeterminate, white cottony growth on agar cylinder (FIG. 2, *B*).

In 6 weeks isolation *71522* consistently produced a white well-defined poroid area on the mycelial pad which formed on slant (FIG. 2, *C*). This fruiting structure produced the capitate incrusted cystidia and the globose spores characteristic of *F. connatus* (3) (FIG. 1, *D* and *E*).

Remarks: F. connatus has been cultured on cornmeal, prune, potato-dextrose and 5 per cent malt agar in addition to regular 1.5 per cent malt agar. The best growth was secured on potato-dextrose and 5 per cent malt. The fungus seems to find conditions on agar media unfavorable and makes slow growth. On sterilized hard maple blocks in large mouth flasks, the fungus grew vigorously, completely covering the blocks with a dense, thick, cottony covering (FIG. 2, *D*). Due to slow growth on malt agar, ordinary cultural methods fail to isolate it satisfactorily from decayed wood

[4] Gallic acid medium is prepared by the addition of 0.5 per cent gallic acid to ordinary malt agar; tannic acid medium by the addition of 0.5 per cent tannic acid. White rot fungi produce a brown diffusion zone under mat. Brown rot fungi form no diffusion zone.

since the presence of bacteria and other fungi results in the slow-growing *F. connatus* being rapidly overgrown.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPT. OF AGRICULTURE,
WASHINGTON, D. C.

LITERATURE CITED

1. **Bavendamm, W.** 1928. Ueber das Vorkommen und den Nachweis von Oxydasen bei holzzerstörenden Pilzen. Zeits. Pflanz. Schutz. **38**: 257–276.
2. **Baxter,** D. V. 1925. The biology and pathology of some of the hardwood heart-rotting fungi. Am. Jour. Bot. **12**: 522–552.
3. **Overholts, L. O.** 1935. Polyporaceae of Pennsylvania. Tech. Bull. 316. The Pennsylvania State College.

EIDAMELLA SPINOSA (MATRUCHOT AND DASSONVILLE) REFOUND

EDWARD D. DELAMATER [1]

(WITH 27 FIGURES)

INTRODUCTION

Matruchot and Dassonville (11) published their account of *Eidamella spinosa* in 1901 for the first and only time that this organism has been reported. In the spring of 1936 Dr. N. F. Conant of the Department of Bacteriology, Duke Medical College, gave to the writer a culture of an obviously gymnoascaceous fungus, which he had cultured from a fingernail while working at the Beth Israel Hospital in Boston in 1935. The fungus was at that time tentatively identified as *Eidamella spinosa* (Mat. & Dass.). Since then the organism has been extensively studied in this laboratory and compared with the very lucid description of the two original authors and with other members of the family Gymnoascaceae. Cultures of the original strain have not been available, but there can be no doubt of its identity with the fungus described as causing a skin condition in a dog in France in 1901, although certain minute differences are noticeable, as will be shown below. These are, however, taken to be within the limits of variation of the form, or as due to differences in observation.

Matruchot and Dassonville considered *Eidamella spinosa* to be a true dermatophyte, although they were unable to reproduce typical lesions by inoculation. They did, however, isolate it more than once from the original lesion. The writer has also been unable to produce typical ringworm lesions with the present cultures, even though he has attempted to inoculate both himself and guinea pigs. The fact, however, remains that this fungus, the two times that it has been found, has occurred in definitely pathological conditions. This fact along with the knowledge that to experimentally induce infection with ringworm fungi is very difficult, can be taken as highly suggestive, though by no means final. The

[1] From the Laboratory for Medical Mycology, Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York City.

question whether *Eidamella spinosa* is truly a pathogen or only a facultative parasite is, then, still an open one.

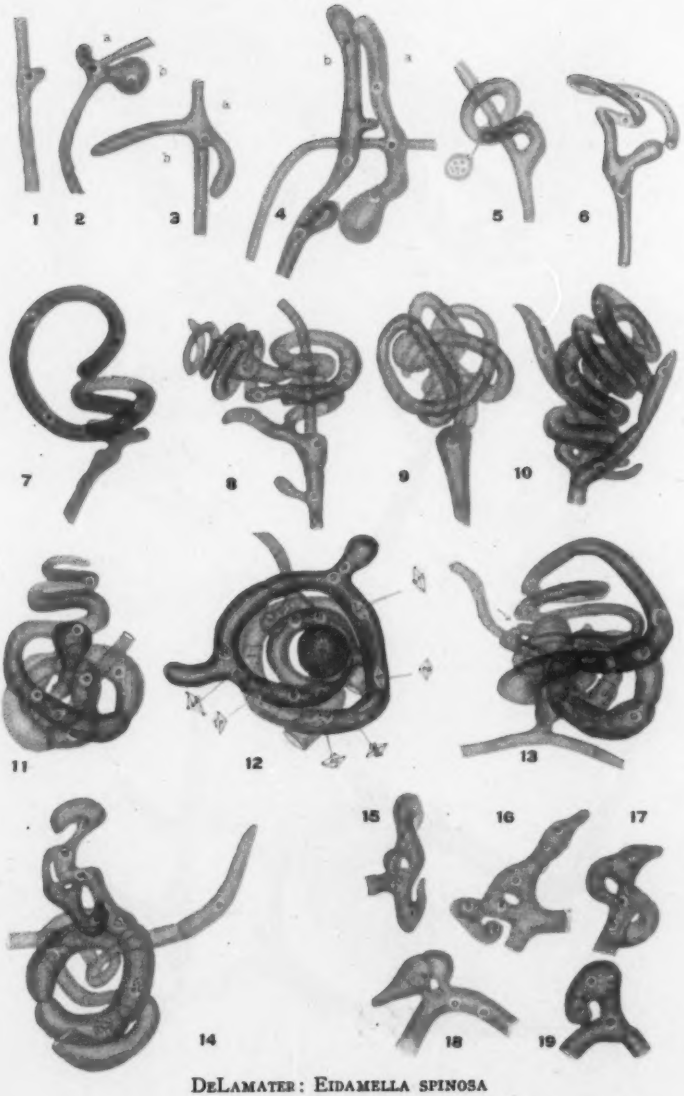
Datta (**4**) reported a sexual organism which he found as a parasite on a dog in India, and with which he was able to repeat infection. The writer has not been able to obtain this account and has had to rely on a second-hand statement given by Gregory (**10**), who had seen only an abstract. The chief difference noted is that Datta's form, *Eidamella actoni,* produces many (more than 8) ascospores per ascus, whereas in *Eidamella spinosa* the ascospore number is constantly eight, as in most of the true Gymnoascaceae, although a very few cases of 4-spored asci have been noted in other forms studied (**5**).

Dr. C. W. Dodge (**6**) has placed *Eidamella spinosa* and *Gymnoascus setosus* as synonyms. The writer from his study of both these organisms in culture and from a preview of previous accounts can find no justifiable evidence to sustain Dodge in his action. Matruchot and Dassonville have elaborately and in most details correctly described their fungus and it compares with *G. setosus* only in the production of spines, which are quite dissimilar in the two organisms, according to the reports in the literature. The writer has as yet not been able to induce sexuality in *G. setosus* in culture, however, the asexual forms are widely dissimilar. There follows a brief comparative summary of the characters of the two organisms obtained from the literature of the older writers. It will be readily seen that these two fungi are not the same. *Gymnoascus setosus* probably lies near the type species, *G. Reessii* Baranetsky, and asexually is very like *G. ruber* from both of which *E. spinosa* is readily distinguishable.

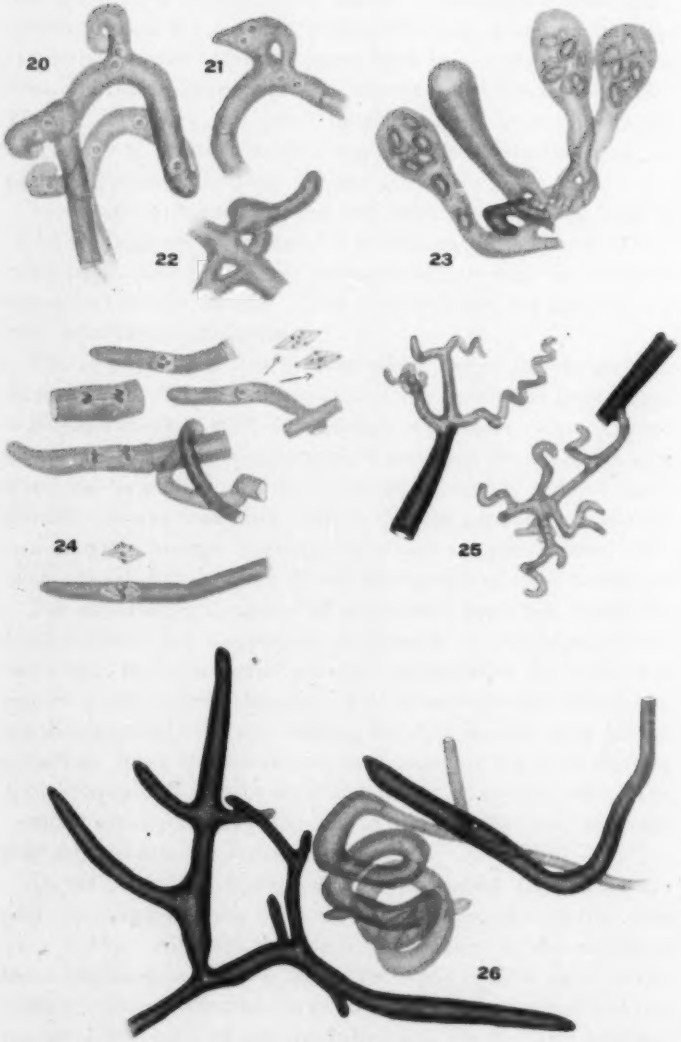
GYMNOASCUS SETOSUS	EIDAMELLA SPINOSA
Peridial hyphae:	*Peridial hyphae:*
a. Thick-walled.	*a.* Thick-walled, strongly cutinized.
b. Dark brown, opaque.	*b.* Blue-black.
c. Branches end in spines.	*c.* Branches do not all end in spines.
	d. Branches form arborescences, forming a trellis about asci. Hyphal tips end in characteristic spirals, especially about asci.
	Some tips fragile, hyaline.

GYMNOASCUS SETOSUS	EIDAMELLA SPINOSA
Asci:	*Asci:*
Spherical, 8-spored, colorless.	3–4 μ wide by 6–7–10 μ long, colorless.
Not pediculate.	Strongly pediculate.
Ascospores:	*Ascospores:*
Spindle-shaped.	Lemon shaped, 1.5 μ by 3 μ.
Colorless.	Colorless.
Form conidia by budding.	Form no conidia by budding.
Hyphae:	*Hyphae:*
Do not anastomose.	Do anastomose freely.
Branch freely, septate.	Much branched, septate.
	In dry culture size variable, 1.5–4 μ.
	In wet cultures size constant.
Conidia:	*Conidia:*
Numerous.	None formed.
Formed in verticillate manner, chiefly in clumps at nodes, often these increase by budding as in *G. ruber.*	No bud cells found.
Chlamydospores.	Chlamydospores
	Not enlarged (intercalary)
	Or enlarged (terminal)
	Form in old cultures by local encystment of ordinary vegetative cells.
Mycelium:	*Mycelium:*
Colorless.	On cornmeal colorless, no pigment formed.
	On potato colorless, no pigment formed.
	On Sabouraud's honey white, pigment formed (blood red).
	On maltose (Sabouraud's) same.
	On blood agar same.
	On all five media the sexual stage appears black or grayish.
Sexual phase: (after Eidam)	*Sexual phase:*
Early coil same as in *Gymnoascus Reessii*, details unknown.	Sexual coil as in *Ctenomyces serratus.*
Sexual stage brownish.	
Fuseaux:	*Fuseaux:*
None formed.	None formed.

The organism: This strain of *Eidamella spinosa* produces perithecia in abundance in all the culture media in which it has been grown, such as Sabouraud's honey and maltose agars, potato, cornmeal, and blood agar, etc. This is not true for the strain of *G. setosus* now in culture.



DeLamater: Eidamella spinosa



DeLamater: Eidamella spinosa

The asexual vegetative growth grossly resembles many of the true dermatophytes. However, microscopically no conidia of any sort are formed on any of the media. Thick-walled intercallary chlamydospores are, however, produced in large quantities, and less regularly terminal chlamydospores have been seen. Ascospores, then, and chlamydospores are the only types of reproductive bodies known. This fact was noted by the original authors. On the contrary in *G. setosus* abundant conidia of the verticillate type are produced, as noted by Dale (**3**) and others.

In culture on honey, maltose, and other agars, a very striking blood-red pigment is produced. It infuses the substrate, often completely coloring it. Only hyphae in contact with the substrate themselves become colored. This is what Matruchot and Dassonville called autopigmentation.

The mycelium is ordinarily pure white, except for the pigment as mentioned, and at the inception of the perithecial stage, when it becomes speckled with a gray-black appearance. On cornmeal and potato media the aerial growth is scant and the perithecia each stand out as minute, definite black specks against a white background. It was from such cultures that the permanent iron-alum haematoxylin mounts, drawings of which are shown here, were made (FIG. 1–26). Figure 27 is a photograph of these perithecia.

The mycelium is composed of abundantly branched, segmented hyphae which vary considerably in diameter in the aerial growth, but which, in the subaerial growth, are composed of hyphae the size of which is quite constant. The intercallary chlamydospores are approximately the same size as the hyphae; are thick walled, cylindric. They are not swollen as in many of the fungi and are formed apparently by the local encystment of hyphal cells. The terminal chlamydospores are not so thick walled and are considerably swollen; they occur in chains.

Sexual phase: Microscopically the perithecial fundiments appear as irregular coils, with or without knoblike central cells (FIG. 1–13). Although there is little regularity in the coiling of these structures in the early stages, the aspect of all is more or less similar. One questionable fusion between the central cell and coil has yet been observed, but the indications are that two adjacent cells of the coil itself fuse and the daughter nuclei from these cells

become associated, and subsequently give rise to ascogenous hyphae by typical crozier formation (FIG. 12, 14–23). These phenomena will be elaborated upon at a later time. Matruchot and Dassonville considered that the stages they observed recalled development of *Ctenomyces serratus* Eidam.

The hyphae forming the peridium of the perithecium arise both from the filament from which the coil originated, from the coil itself, and from the vegetative aerial hyphae surrounding the primordium of the future perithecium. The perithecium, then, is of compound origin. These peridial hyphae are easily recognizable, even in their very early stages, by their very characteristic shape and density. As they gradually reach maturity they become thick walled and of a dense blue-black under the microscope (FIG. 3, 4, 14, 24, 25, 26). They form about the young, developing coil a weft of closely entwined hyphae, the outer extremities of which later become definitely spined (FIG. 26). Directly surrounding the ascogenous mass the branches of the thick, black protective hyphae end in clear hyaline coils instead of spines (FIG. 25). These vegetative peridial coils branch irregularly and form a dense protective trellis directly surrounding the ascogenous mass (asci). This multiple structure is very characteristic of the older perithecia, and form about the time the asci begin to enlarge. Some of the short lateral branches of these peridial hyphae do not form either spines or coils, but are thin walled, hyaline, with dense cytoplasm, and are very fragile. They seem to be growth centers by which the peridial hyphae increase in extent and complexity. They simulate closely the condition found in the rapidly growing younger stages, in which the walls have not yet begun to thicken.

The asci themselves are longly pediculate, and each contain eight oval spores. Both asci and spores are hyaline. The asci are more strikingly pediculate in this strain than in that studied and figured by the original authors for theirs, but they emphasized the presence of the condition and figured it. At rupture of the ascus the eight spores often adhere, apparently due to a cohesive mucilage, which holds them together.

Thirteen single ascospore cultures all produced perithecia in

which normal spores were found. This very definitely indicates that this fungus is hermaphroditic and self-fertile (homothallic), which has an interesting significance when one considers the work of Emmons (**9**) and DeLamater (**5**) in which nineteen species of Penicilliceae and three of the Gymnoascaceae were found to be hermaphrodite and self-fertile.

In this paper it is not desired to give a complete story of the development of this fungus, but rather to note that this interesting organism, which was so strongly emphasized by its original describers as a sexual form of one of the skin fungi, has been refound and that although it has not as yet been proven pathogenic, it was at least, as in the original case, found in a definitely pathological condition. Whether it was the causative agent or not is another question, which is as yet unsettled. To date the animal inoculation work with the ringworm fungi has been largely unsatisfactory, and until a criterion of infectivity or infectiousness is obtained it will be difficult to establish not only this case but many which are today considered offhand as pathogenic.

The distribution of this fungus, the two times that it has been found, presents of itself an interesting problem. That the same fungus should be found first in France in a pathological condition, and then thirty-four years later, also in a more or less similar pathological condition, but several thousand miles from the original place of discovery, is certainly worthy of note, though at the present time far from being explained. Unfortunately nothing is known concerning the mode of life, origin, or travels of the patient involved.

The refinding of *Eidamella spinosa* rejuvenates an old controversy concerning the possibility of sexuality among the ringworm fungi. It also reopens the old question of placement of these pathogenic organisms among lower Ascomycetes in the family Gymnoascaceae, instead of in the Fungi Imperfecti where they are now usually considered. Matruchot and Dassonville in their presentation of the belief that the dermatophytes belonged to this family of ascomycetes were rather dogmatic, and many workers including Sabouraud at that time considered that there was insufficient data then available for the final placement of these organisms here. Since then attempts have been made to do so,

although the state of knowledge today is much as it was in 1901 on this point.

Several reports of ascospore formation have appeared in the literature since that time. The report of the occurrence of asci in *Trichophyton Currii* by Chalmers and Marshall (**1**), with due regard for these workers, requires a comprehensive restudy of this organism and its coincidental comparison with various known sexual forms before it can be accepted.

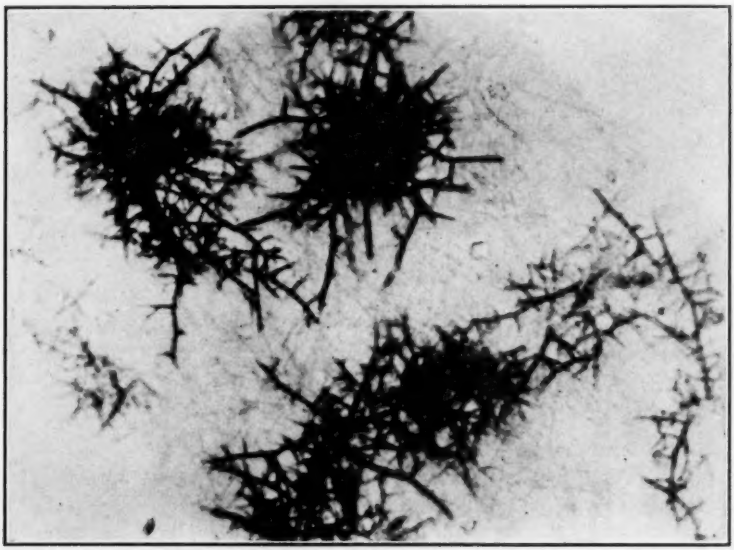


FIG. 27. Perithecia in *Eidamella spinosa.*

The brief report of *Gymnoascus gypseum,* published by Nannizzi (**13**) in 1927 in which he described asci has been severely criticised for lack of conclusive evidence. It is regrettable that Nannizzi did not produce a more comprehensive analysis with figures which could be used in comparison and identification.

If it is possible to obtain a culture of the organism described by Datta (**4**) for comparative purposes, it is hoped that an eventual understanding of the placement of this fungus, which seems at the moment to be the only acceptable case of a pathogenic fungus

producing perithecia yet reported, may be included in the final treatise of the family to be presented at a later time.

It is impossible to claim at the present that the refinding of *Eidamella spinosa* is conclusive evidence to the further establishment of the ringworm fungi as members of the family Gymnoascaceae, despite its cultural similarities and the similarities of the early sexual stages to nodular organs, etc. It is merely another link in a chain of evidence. The writer can find as yet no justification for the reclassification of the dermatophytes among the Gymnoascaceae, as done by Langeron and Milochevitch, Nannizzi, and others, even though the authors can cite morphological similarities between such forms as *Ctenomyces serratus* and certain of the true dermatophytes. Such a step must eventually be based upon the actual finding or inducement of bonafide sexual stages in an unquestionable pathogen; it must also bear repetition. The present case is the only one to date which can claim this last point of eminence, and to the mind of the present writer even this does not make it a sufficiently clear-cut case upon which to base a modified taxonomy.

From the purely mycological point of view it would be both interesting and valuable to know whether the ringworm fungi are members of the Gymnoascaceae. It would, however, of necessity be of purely theoretical interest and of very little practical value unless perithecia could be easily induced in all or most of the dermatophytes met with clinically; which is very unlikely. The practical handling and identification of these would still be as difficult as before.

As a matter of fact from the point of view of an individual attempting to learn something of the vagaries of these fungi, the classification presented by Sabouraud in 1910 has been neither added to nor elucidated, either by the attempts to place them in the Gymnoascaceae or by the numerous attempts to reclassify them by means of very variable botanical characteristics.

It is readily seen that until some more constant, easily recognizable, and usable criteria are found for the taxonomic diagnosis of these fungi, the present studies based on morphology, interesting as they are, add little to the clarification of this problem, though upon close analysis they may give some notion as to the limits of

variation of individual species, whatever species may eventually turn out to be.

With this in mind the refinding of *Eidamella spinosa* (Matruchot and Dassonville), found for the second time in 34 years in a pathological condition, but itself of questionable pathogenicity, is presented for whatever value and interest it may have from the point of view of those with a purely mycological interest.

To Professor J. G. Hopkins, Dr. R. W. Benham and Dr. B. O. Dodge I wish to express thanks for their interest and helpful suggestions. To Dr. N. F. Conant I wish to express my gratitude for turning over the cultures of *Eidamella spinosa* to me and thus making this and future studies on this form possible.

BIBLIOGRAPHY

1. **Chalmers, A. J. & Marshal, A.** 1914. The systematic position of the genus *Trichophyton* Malsten 1845. Jour. Trop. Med. (Hyg.) **17**: 289.
2. ——. 1914. *Tinea capitis tropicalis* in the Anglo-Egyptian Sudan. Jour. Trop. Med. & Hyg. **17**: 257.
3. **Dale, E.** 1903. Observations on the Gymnoascaceae. Ann. Bot. **17**: 571.
4. **Datta, S.** 1932. A new fungus, *Eidamella actoni*, parasitic to the dog. Indian Vet. Jour. **8**: (Cited from Gregory.)
5. **DeLamater, E. D.** 1937. Crozier formation in the Gymnoascaceae. Mycologia **29**:
6. **Dodge, C. W.** 1935. Medical Mycology, p. 900. Mosby Co. St. Louis.
7. **Eidam, E.** 1883. Jahresbericht der Schlesischen gesell. 175.
8. ——. 1882. Botanische Centralblatt **10**: 107.
9. **Emmons, C. W.** 1935. The ascocarps in species of *Penicillium*. Mycologia **27**: 128.
10. **Gregory, P. H.** 1935. The Dermatophytes. Biol. Rev. Cambridge Phil. Soc. **10**: 2.
11. **Matruchot, L. & Dassonville, C.** 1901. *Eidamella spinosa*, Dermatophyte produsiant des Peritheces. Bull. Soc. Myc. Fr. **17**: 123.
12. ——. 1889. Sur le *Ctenomyces serratus*, Eidam compare aux champignons des teignes. Ibid. **15**: 305.
13. **Nanizzi, A.** 1927. Ricerche sull'origin saprofitica dei funghi delle tigne. II. *Gymnoascus gypseus* (n. sp.), *Sabouraudites* (*Achorion*) *gypseum* (Bodin) Ota et Langeron. Inst. Bot. Roy. Univ. Siena.
14. ——. 1926. Ricerche sui rapporti morphologici e biologici tra Gymnoascaceae de Dermatomiceti. Ibid. and Ann. Myc. **24**: 85.

EXPLANATION OF FIGURES

All drawings were done with the aid of a camera lucida, × 1100.

Fig. 1. Very young sexual coil arising from parent hypha. The uninucleate origin of the future nuclei of the coil is apparent.

Fig. 2. Slightly older stage showing the sterile, uninucleate central cell and a young coil, both arising from the same hypha.

Fig. 3. Slightly older coil (*a*) arising from same hypha from which a primordial protective (*b*) or peridial hypha is arising. The nucleus in the parent hypha has not yet divided, thus giving rise to the nuclei of each type of branch.

Fig. 4. (*a*) Young coil showing uninucleate condition before the basal wall has been formed. The sterile central cell arises from the opposite side of the same parent hypha. (*b*) Protective hypha which has arisen from the same parent hypha (out of the field) and which has become associated with the sexual structures.

Fig. 5. Somewhat older coil in the uninucleate condition before the basal wall has come in. A polar view of the nucleus in metaphase is shown enlarged to one side.

Fig. 6. Shows the 3-nucleate stage of the coil. The basal wall has been cut off. A sterile central cell is seen to arise from the parent hypha at the base of the coil.

Fig. 7. Older sexual coil showing segmentation into uninucleate cells. No central cell is evident.

Fig. 8. Older, more complex sexual coil wrapping about both the parent hypha and the sterile uninucleate central cell. No fusion between the coil and the central cell can here be seen. A sterile protective hypha is arising from the base of the coil; another is arising from the parent hypha.

Fig. 9. Very complex coil. The sterile central cell lies more or less in the center of this maze, unfused with any part of it.

Fig. 10. Somewhat later stage in which the sterile, unfused central cell; the coil; and protective branches arise from the same parent hypha or complex. Note that the peridial branches very soon after their appearance assume a very characteristic form which is easily recognizable.

Fig. 11. Coil and central cell arise from same parent hypha. No fusion between the central cell and the coil is evident. Note the enlarged size of the uninucleate cells of the coil.

Fig. 12. Very important stage. The origin of the binucleate condition of the cells of the coil which give rise to the croziers is indicated by the mitoses shown in the coil. The actual formation of binucleate crozier fundiments is seen. The sterile central cell which has arisen from a separate source from that of the coil is seen to be uninucleate and unfused with any part of the coil. The mitotic figures are numerous, and the indications are that there are five chromosomes. It is extremely difficult to be sure of an accurate count because of the very small size of the nuclei.

Fig. 13. Shows a very complex coil. It is seen here that the tip of the coil seems to fuse with the central cell. Due to the complexity of the structure it was very difficult to determine this with certainty. It is presented as a possible case of fusion only. It has been observed also by Dr. Rhoda Benham and others and was considered as questionable by them also.

Fig. 14. Sexual coil showing croziers arising from it. Nuclei are migrating to appropriate position for conjugate division in crozier and are in what appears to be early prophase.

Figs. 15, 16, 17, 18, and 19 are various stages in the development of the croziers. The lower hook process in No. 15 shows essentially what is seen in Fig. 14. Figure 17 shows the fusion of the tip cell of the crozier with the basal cell in the reestablishment of the binucleate condition. The young ascus is seen to be binucleate at this stage. Figure 19 shows the young ascus formed directly after the conjugate division. Here the fusion of the tip cell with the basal cell of the crozier has not yet occurred. Figure 18 shows a young ascus arising from a crozier which arose directly from the coil. It is seen that nuclei in this young ascus are in the process of fusing, thus establishing the diploid nucleus of the ascus, which is later reduced at spore formation. Such a meiosis is here postulated, but has not as yet been observed in *Eidamella spinosa*.

Fig. 20 and 21. Other stages in crozier formation.

Fig. 22. Shows the young ascus elongating, thus approaching its mature shape.

Fig. 23. Shows three fully mature asci with their included ascospores. Eight ascospores are usual per ascus, in fact no other number has ever been observed. Note also the dipodial bases of the asci, indicating their crozier origin. A young crozier and an ascus in which no spores have yet been cut out are shown.

Fig. 24. Mitotic figures observed in developing peridial hyphae. Prophase, metaphase, and telaphase are shown. Such figures as these also indicate a probable haploid chromosome number 5, possibly 4, 6, or 7. They are much too small to be made out with certainty.

Fig. 25. Typical spirals which occur at the tips of peridial branches closely surrounding the cluster of asci in the center of the loose, arachnioid perithecium. Note the blackish, thick-walled cells from which they arise.

Fig. 26. Young sexual coil with dark, thick-walled peridial hyphae being differentiated around it from the surrounding aerial hyphae, which, other than their proximity have no direct connection with the sexual coil itself.

Thanks are tendered to Mr. Alfred Feinberg for his helpful suggestions in the execution of these drawings; and to Dr. Rhoda Benham for her thoughtful observation of many of the structures here drawn.

Fig. 27. Photomicrograph of perithecia in cornmeal agar culture of *Eidamella spinosa* (low power).

MISCELLANEOUS NOTES ON THE USTILAGINALES [1]

GEORGE L. ZUNDEL

(WITH 1 FIGURE)

This paper is a record of miscellaneous observations on the Ustilaginales as follows,

I

Thecaphora leptideum (Syd.) Zundel, comb. nov.

Tolyposporium leptideum Syd. Ann. Myc. **11**: 365. 1913.

In 1913 Sydow described a smut on *Chenopodium album* L. collected at Forbach, Lorraine, France to which he gave the name of *Tolyposporium leptideum* and published the following description:

"Soris in ovariis leniter quam normales majoribus evolutis, matricem leniter deformantibus, cinnamomeo-brunneis; glomerulis rotundatis 35–50 μ diam., vel rarius ellepticis aut oblongis et tunc usque 60 μ longis, ex sporis numerosis denissime coalitis compositis; sporis singulis angulato-globosis, angulato-ovatis vel cuneatis, superficie verruculosis, ad latera levibus, dilute brunneis, 9–16, 8–12 μ, membrana ca. 1 μ crassa."

Besides the type specimen, material collected near Kreuzeburg, Germany, Hungary, and Brno, Czechoslovakia, has been examined. It was noted that the spore balls are yellowish in color. According to the usually accepted classification, all smuts with yellowish or reddish colored spore balls are referred to the genus *Thecaphora*. The spore balls of the genus *Tolyposporium* are always dark colored. The name of this smut then should be *Thecaphora leptideum* (Syd.) Zundel.

In a collection of unnamed specimens of smuts I found one that had been sent to me from New South Wales and labeled "*Soro-*

[1] Contributions from the Department of Botany, The Pennsylvania State College, No. 99.

sporium sp. on *Chenopodium ambrosioides* L., Coll. 1914, N.S.W." The name of the collector is not given. An examination of this specimen revealed that it was *Thecaphora leptideum*. This is apparently the first report of this smut from Australia and also the first report outside of Central Europe.

SOROSPORIUM CONSANGUINEUM Ellis & Ev. Jour. Myc. **3**: 56. 1887.

Ustilago Aristidae Peck, Bull. Torrey Bot. Club **12**: 35. 1885.

Sorosporium Bornmülleri P. Magn. Ver. Zool.-Bot. Gesell. (Wien) **50**: 434. 1900. (Not *S. Aristidae* Neger, Anal. Univ. Santiago (Chile) **93**: 789. 1896.)

In 1885 Peck described a smut on "*Aristida Rusbyi*" (*A. arizonica*) to which he gave the name *Ustilago Aristidae*. Later it was found that this smut was a *Sorosporium* and in 1887 Ellis and Everhart renamed the smut *Sorosporium consanguineum*. In 1900 P. Magnus described a *Sorosporium* on *Aristida coerulescens* Desf. from Syria which he named *Sorosporium Bornmülleri*. The Magnus species was later collected in 1931 by P. Unamuno in Morocco on *Aristida coerulescens* Desf. A comparison of the specimen of *S. Bornmülleri* collected in Morocco and specimens of *S. consanguineum* collected in different parts of the world reveals that both species are identical. The name *S. Bornmülleri* must therefore be relegated to synonymy.

II

The following smuts were collected by Dr. A. S. Hitchcock in 1929 during a trip to Africa to collect grasses. Most of the hosts are apparently new for the smut species.

1. USTILAGO CYNODONTIS P. Henn. On *Cynodon plectostachyum* (K. Schum.) Pilger, Kampala to Jinja, Uganda, Sept. 18, 1929, No. *24951*.

2. USTILAGO DIGITARIAE (Kunze) Winter. On *Digitaria abyssinica* (Hoch) Stapf, Kisumu, Kenya, Sept. 15, 1929, No. *24844*; on *Digitaria* sp., Entebbe, Uganda, Sept. 16, 1929, No. *24877*.

3. USTILAGO RABENHORSTIANA Kühn. On *Digitaria horizontalis* Willd., Nairobi to Nanyuki, Kenya, Sept. 1929, No. *24764*.

4. **Ustilago Hitchcockiana** Zundel, sp. nov.

Sori entirely destroying and deforming the inflorescence, linear, usually 2–7 cm. long, at first concealed by the glumes, covered with a brown membrane which flakes away revealing a dark brown spore mass; spores globose—subglobose, light olivaceous, 3.5–5.5 μ diam., rarely 7 μ, smooth, guttulate.

Soris linearibus, saepissime 2–7 cm. longis, inflorescentiam ex toto destruantibus, et deformantibus, primo in glumis inclusis, membrana brunnea tectis, eaque rupta pulverem sporarum fuscam ostensis; sporis globosis vel subglobosis, pallide olivaceis, 3.5–5.5 μ raro 7 μ diam., levibus guttulatis.

On *Cynodon Dactylon* (L.) Pers., Nairobi, Kenya, Sept. 26, 1929. A. S. Hitchcock No. *25142*.

Three species of smuts have been previously reported on species of *Cynodon* as follows:

1. USTILAGO DREGEANA Tul. Ann. Sci. Nat. III. **7**: 83. 1847.

Type host and locality: On a grass. Cape of Good Hope, Africa.

This species has globose-subglobose spores, olivaceous brown, 4–5 μ diam., indistinctly and sparsely papillate.

2. USTILAGO CYNODONTIS P. Henn. Bot. Jahrb. (Engler) **14**: 369. 1891.

Type host and locality: On *Cynodon Dactylon* (L.) Pers., Abyssinia, Africa.

This species has globose-subglobose (or occasionally ellipsoidal) spores, olivaceous, 7–10 μ diam., smooth, granular (some writers describe this species as almost smooth).

3. USTILAGO PARAGUARIENSIS Speg. Anal. Soc. Cien, Argentina **17**: 89. 1884.

Type host and locality: On *Cynodon dactylon,* Paraguay.

This species is described as having globose spores, dark olivaceous, 7–9 μ diam., granular pappilulate.

None of the described species have spores identical to *Ustilago Hitchcockiana. Ustilago Dregeana* Tul. has spores about the same size as *Ustilago Hitchcockiana* but differs in having a papillate epispore. The writer has not been able to locate an authentic specimen of *Ustilago Dregeana* Tul.

5. **Sphacelotheca Kenyae** Zundel, sp. nov.

Sori destroying the inflorescence, about 1 cm. long, concealed by the glumes, covered by a delicate brown membrane which flakes away revealing a semi-agglutinated mass of spores surrounding a columella; sterile cells globose-ellipsoidal, usually regular, hyaline, 12–17 μ diam., usually in pairs; spores globose-subglobose, olivaceous with a thick epispore, usually 9–11 μ diam., smooth non-granular.

Soris circa 1 cm. longis, inflorescentiam destruantibus, in glumis inclusis, membrana tenue brunnea tectis, eaque rupta pulverem sporarum semi-agglutinatarum columellam amplectentium ostensis; cellulis sterilibus globose-ellipsoideis, saepissime geminatis; sporis globosis vel subglobosis, olivaceis, saepissime 9–11 μ diam., nongranularibus, episporio crasso, levi.

On *Hyparrhenia* sp. Eldoret, Kenya, Sept. 20, 1929, Coll. A. S. Hitchcock, No. *25028*.

This species is closely related to *Sphacelotheca Ruprechtii* Syd. The size and color of the spores of the two species is identical. *Sphacelotheca Ruprechtii* differs from *Sphacelotheca Kenyae* by having smooth granular spores, smaller sterile cells and a larger sorus.

6. TILLETIA HETEROSPORA (P. Henn.) Zundel (*Ustilago heterospora* P. Henn.; *Tilletia Ayresii* Berk.).

On *Setaria aurea* Hochst., Jinja, Uganda, Sept. 18, 1929. No. *24962*.

III

Through the kindness of Dr. W. Robyns, Director of the Jardin Botanique de l'Etat, Bruxelles, Belgium, eleven specimens of smuts collected in the Belgian Congo by Hyac. Vanderyst, missionary, were loaned for study. Most of them are type specimens used by P. Hennings for the original descriptions. On the sheets are the original notations and pencil drawings made by P. Hennings. In general, the specimens contain few spores and fewer sori. The result of examining the specimens is as follows:

USTILAGO BECKERAE P. Henn. Ann. Mus. Congo V. **2**: 86. 1907.

An examination of the type specimen fails to show the presence of any smut spores although P. Hennings has pencil drawings of apparently typical *Ustilago* spores attached to the sheet. Appar-

ently the supply of smut spores was very limited and all of them have been used in study. Since this smut has been collected only once, it seems best to regard *Ustilago Beckerae* as a doubtful smut until more material is collected. One spikelet resembling a sorus of a *Ustilago* is in a small packet in the upper left hand corner of the sheet but an examination under the microscope showed it to be a dark spored species of the Fungi Imperfecti. This material is also the type for *Plascosphaeria Beckerae* P. Henn.

On *Beckera* sp., Kisantu, Congo, May 1906, Coll. Vanderyst, No. *169*.

USTILAGO SETARIAE-AUREAE P. Henn. Ann. Mus. Congo V. **2**: 86. 1907.

An examination of the type specimen shows that the original description can be rewritten as follows:

Sori destroying the ovaries, inflated; spores subglobose, irregular, olivaceous brown, 3.5–5 μ diam., verruculate to tuberculate.

On *Setaria aurea* Hochst., Dembo, Congo, June 1906, Coll. Vanderyst, No. *B28*.

USTILAGO ISCHAEMOIDES P. Henn. Ann. Mus. Congo V. **2**: 86. 1907.

An examination of the type specimen reveals the presence of spore balls. This character places the species in the genus *Sorosporium*. A redescription is as follows:

Sorosporium ischaemoides (P. Henn.) Zundel, comb. nov.

Sori destroying the inflorescence, horn shaped, about 1 cm. long, covered by a thick yellowish colored membrane which dehisces apically revealing a granular spore mass surrounding a simple columella; spore balls opaque, many spored, subglobose-ellipsoidal, irregular, semi-permanent, reddish brown, 50–105 μ long; spores subglobose-ellipsoidal, dark olivaceous brown (spores on the interior of spore balls, lighter colored), thick epispore, about 2 μ thick, 7–10 μ diam., smooth.

On *Andropogon* sp., Leopoldville, Congo, May 1906, H. Vanderyst, No. *121*.

Attached to the specimen is the original description apparently in the handwriting of P. Hennings.

CINTRACTIA CONGENSIS P. Henn. Ann. Mus. Congo V. **2**: 87. 1907.

Sori in the axiles of the stem whorls and extending up on the side branches, agglutinated, at first covered with a delicate membrane; spores globose-ellipsoidal, regular, dark olivaceous brown, 7–10 μ diam., smooth and somewhat granular.

On an unidentified species of the Cyperaceae, at the side of a path between Matada and Leopoldville, Congo, May 19, 1906, Coll. H. Vanderyst, No. *105*. This is the type specimen.

The original description describes the spores as angular. An examination of the type specimen did not show this character and furthermore the original drawings by P. Hennings do not show angular spores.

CINTRACTIA TOGENSIS P. Henn. Bot. Jahrb. (Engler) **38**: 119. 1905–1907.

Sori destroying the inflorescence, ellipsoidal, hard crusted but spores wear off producing a powdery mass, about 1 cm. or less; spores globose—broadly ellipsoidal, regular, occasionally with hyaline wings, light olivaceous brown, not opaque, 10–14 μ diam., smooth.

On an undetermined species of the Cyperaceae, Kisantu, Congo, May 1906, Coll. H. Vanderyst, No. *126*.

The specimen examined is not the type but it was determined by P. Hennings.

CINTRACTIA VANDERYSTII (P. Henn.) Zundel, Mycologia **22**: 128. 1930.

Ustilago Vanderystii P. Henn. Ann. Mus. Congo V. **2**: 86. 1907.

Sori destroying the basal parts of inflorescence, long linear, 5–10 mm. long, concealed by the glumes, at first covered by a tawny colored membrane; spore masses agglutinated around a central columella; spores subglobose-ellipsoidal, fragile, olivaceous brown, 7–10 μ diam., smooth but granular.

On *Andropogon* sp., Kisantu, Congo, Coll. H. Vanderyst, 1908.

The specimens examined were determined by P. Hennings but were not type specimens.

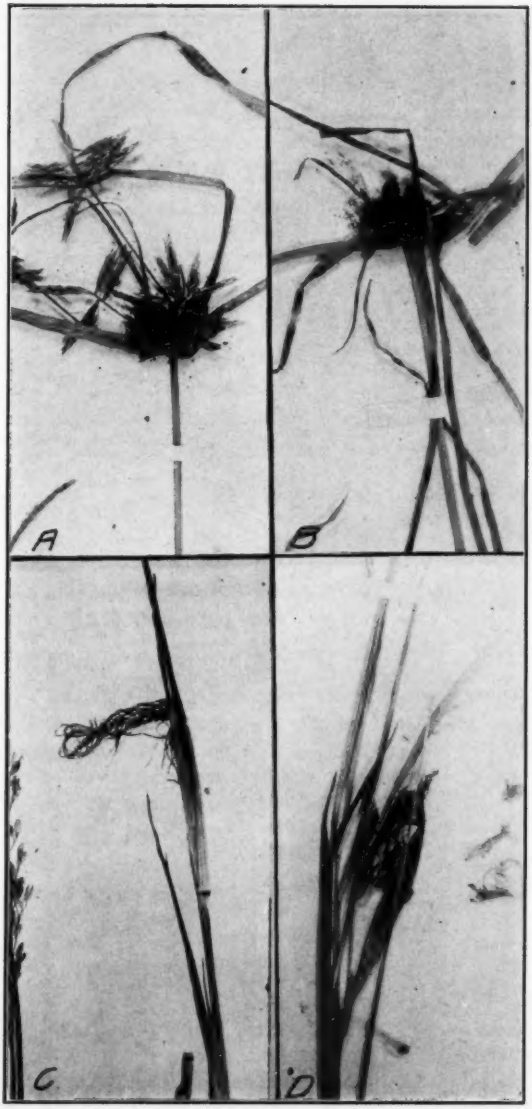


FIG. 1. A, *Cintractia congensis* P. Henn.; B, *Cintractia togensis* P. Henn.; C, *Sorosporium kinshaensis* (Beeli) Zundel; D, *Sorosporium Wildemanianum* P. Henn.

SOROSPORIUM PANICI Beeli, Bull. Jard. Bot. Brux. **8**: 7. 1923.

An examination of the type specimen reveals the presence of two fungi neither of them a *Sorosporium*. One of them was found to be a *Cladosporium*-like fungus, the other is a *Ustilago*, near or identical to *Ustilago ugandensis* P. Henn. In the absence of authentic material of *U. ugandensis*, this determination is not final. The drawings intended for *Sorosporium* spore balls are not typical for this genus.

Even if this was a *Sorosporium* the specific name "*Panici*" could not be used here since in 1912 MacKinnon used the binomial *Sorosporium Panici* for another smut found in Australia. Previous to having this specimen available for examination the author had changed the name of the Beeli material to *Sorosporium Beelii* in a paper now gone to press.

SOROSPORIUM PANICI var. KINSHASAENSIS Beeli, Bull. Jard. Bot. Brux. **8**: 8. 1923.

An examination of the type specimen shows that this smut is not related in any way to the species of which it is supposed to be a variety. It is therefore proposed to raise the variety to specific rank with the following description:

Sorosporium kinshasaensis (Beeli) Zundel nom. nov.

Sori destroying the inflorescence, 3–5 mm. long, at first concealed by the glumes, covered by a membrane which flakes away revealing a granular spore mass intermixed with brown shreds; spore balls usually ellipsoidal, somewhat irregular, opaque, many spored, permanent; spores globose-subglobose, irregular, reddish brown, fragile, 5–8 μ diam., smooth.

On *Panicum kinshasaënse* Vanderyst, Kinshasa, Congo, June 2, 1916, Coll. H. Vanderyst, No. *4*.

SOROSPORIUM WILDEMANIANUM P. Henn. Ann. Mus. Congo V. **2**: 87. 1907.

Sori destroying the inflorescence, long linear, 1–1.5 cm. long, grouped, covered with a thick smooth membrane which ruptures apically revealing a granular spore mass intermixed with long dark shreds; spores subglobose-ellipsoidal, somewhat angular, not fragile, olivaceous with a thick reddish brown epispore about 1.5 μ thick, 7–11 μ diam., verruculose on exposed surface, otherwise smooth.

On *Andropogon Gayanus* Kunth., Dembo, Congo, June 3, 1908, Coll. H. Vanderyst.

In the specimen examined the spore balls had entirely disintegrated. The original description describes them as "glomerulis ellipsoideis vel subglobosis e sporis numerosis compositis, 50–80 × 50–60 μ."

The author in 1930 (Mycologia **22**: 146. 1930) published a description of this species which states that the sori were on the leaves. Evidently the material used has not been correctly named although it was marked *S. Wildemanianum* and was sent as a correct determination. The specimen examined for the present paper has been previously determined by P. Hennings and is considered correct.

PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PENNSYLVANIA

EXPLANATION OF FIGURE

Congo smuts collected by H. Vanderyst and deposited in the Herbaier du Botanique de l'Etat, Bruxelles.

A. Cintractia congensis P. Henn.
Herbier de Hyac. Vanderyst, No. *105*. Photo of type specimen.

B. Cintractia togensis P. Henn.
Herbier de Hyac. Vanderyst, No. *126*. Photo not type specimen but determined by P. Hennings.

C. Sorosporium kinshaensis (Beeli) Zundel.
Herbier du Jardin Botanique de l'Etat, Bruxelles, Leg. H. Vanderyst. Photo of type specimen.

D. Sorosporium Wildemanianum P. Henn.
Herbier du Jardin de Botanique de l'Etat, Bruxelles, Coll. H. Vanderyst, 1908. Photo not type specimen but determined by P. Hennings.

NOTES ON THE GENUS MICROMYCES

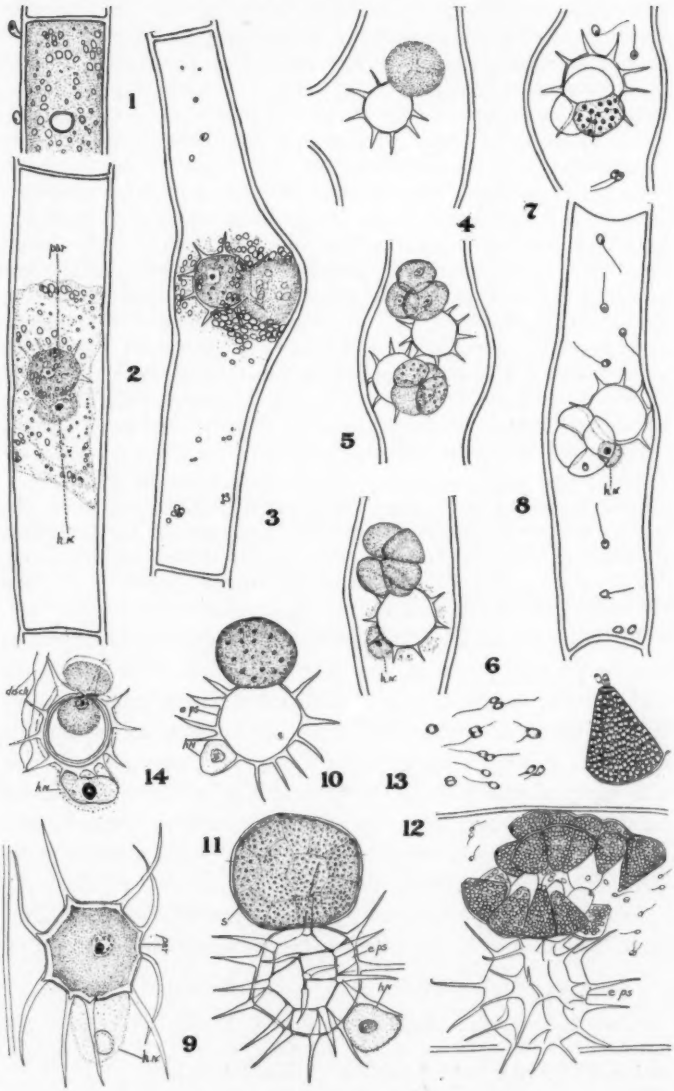
JOHN N. COUCH

(WITH 14 FIGURES)

In 1931 the writer described the life history of a species of *Micromyces* from Long Island, N. Y., which he at that time thought was *M. Zygogonii* Dangeard. Although there were certain distinct differences between the American fungus and the European, it seemed best at the time to consider the two as the same. Since then, however, we have twice collected at Chapel Hill, N. C., in large quantity, a fungus which agrees in all respects with the European *M. Zygogonii* Dangeard as understood by Heidt (**1937**) in his recent detailed treatment of this species. Fortunately three excellent slides of the Long Island parasite have been preserved and hence could be compared first hand with the Chapel Hill fungus. Such comparison has shown beyond doubt that the two are very distinct from each other. The Long Island plant is also quite distinct from *M. Spirogyrae* Skvortzow (**1927**) and *M. Petersenii* Scherffel (**1926**). It is therefore necessary to give the Long Island plant a new name and since it has by far the longest spines of any of the species of the genus the name *longispinosus* would seem appropriate. Since a rather complete account of this form was given by the writer (**1931**) including the fusion of planogametes, only a short description will be repeated here.

Micromyces longispinosus sp. nov. (*Micromyces Zygogonii* Dang. sense of Couch, 1931, but not Dangeard or Heidt, 1937).

Thallus at first a naked, amoeboid, plasma body within the algal cell. At length coming to rest next to the nucleus of the host and growing at the expense of the latter; forming at maturity a more or less spherical body, the prosorus, which contains a single large nucleus up to 8 μ thick. Prosori 10–33.6 μ thick, most about 21 μ thick. Covered by a hyaline, spiny membrane or rarely the membrane may be nearly smooth. Spines up to 22 μ long, most about 12 μ long. The number of spines varies from about 12–24.



FIGS. 1–8. *Micromyces Zygogonii.*
FIGS. 9–14. *Micromyces longispinosus.*

Membrane irregularly reticulated between the spines. The prosorus may germinate directly, discharging its contents into a vesicle, the contents of which now cleave into from about 8 to 24 or even more sporangia. Sorus globose or ovoid, remaining attached to the empty prosorus until after the sporangia are formed, and then losing its shape more or less completely by the swelling and bursting apart of the sporangia. Sporangia usually polygonal in shape, about 8.4 × 9.6 μ thick in their two greatest dimensions; sometimes pyramidal in shape, but with a rounded base and a truncated apex. The contents of the sporangia quickly divide into many, very small, uniciliate zoöspores. Zoöspores subglobose or ovoid with a glistening droplet at one or both ends, about 1 μ thick. Planogametes (?) also formed which apparently fuse to form zygotes. The thallus may become transformed into a pale yellowish brown and thick-walled, uninucleate resting body, which is covered with a hyaline spiny membrane; resting bodies 16–21 μ thick, discharging their contents into a vesicle. The contents later perhaps cleave to form sporangia.

Thallus intra cellulas Spirogyrae parasiticus, primo nudus. Prosori 12–24 numero, 10–33.6 μ diam., plerique 21 μ, membrana aculeata, spinis ad 22 μ longis, plerisque 12 μ longis. Prosorus sorum generans, qui in 8–24 vel plura sporangia dividitur. Sporangia 8.4 × 9.6 μ. Zoosporae admodum parvae, fere 1 μ diam., uniciliatae. Sexuales cellulae etiam formatae. Sporangia perdurantia 16–21 μ.

Found in the cells of *Spirogyra* sp. in a small pond near Cold Spring Harbor, L. I., N. Y., August 7, 1925. The species of *Spirogyra* appears to be near *S. adnata,* but since the alga was only in the vegetative condition, I could not be sure of its identity. The cells measured 37–44 × 147–190 μ; without replicate ends. Chlorophyll bands 3 or 4 making about 2 complete turns. In the same material there was another species of *Spirogyra,* slightly smaller and with only one chlorophyll band. This species was entirely untouched by the parasite.

The present species is very distinct from *M. Zygogonii* Dangeard as indicated in the tabulated comparison below.

MICROMYCES LONGISPINOSUS	M. ZYGOGONII Dangeard
Prosori 10–33.6, most about 21 μ thick	Prosori 11–16.8, most about 13 μ thick
Spines 10–22 μ long, most about 12 μ long	Spines 4–5.5 μ long
Sporangia 8–24 in each sorus	Sporangia 4–6 in each sorus
Spores about 1 μ thick	Spores about 2 μ thick
Resting bodies 16–21 μ thick	Resting bodies about 12 μ thick

MICROMYCES ZYGOGONII Dangeard.

Thallus parasitic in the cells of *Mougeotia, Zygogonium,* and other Zygnemales. At first amoeboid, usually coming to rest within the host cell near the nucleus. Sometimes the parasite may develop not in contact with host nucleus. Forming at maturity a spherical, spiny body, the prosorus. During the development of the prosorus the host cell usually swells considerably, sometimes to twice its normal diameter, sometimes even bursting as a result of the internal pressure, and the protoplast becoming more or less disorganized. Usually one prosorus to a host cell but sometimes several may be formed. Prosorus 11–16.8 μ thick, with 14–18 sharply pointed hyaline spines which are 4–6 μ long. Mature prosorus with a single large nucleus. At maturity the prosorus germinates, emptying its entire contents in a spherical mass which becomes cut up into 4–6 sporangia. Sorus spherical at first, irregular in outline after the sporangia have been formed, 13–18 μ thick. Sporangia irregular in shape, the inner walls are flattened by pressure while the outer wall is rounded. Spores rounded or ovoid, with one posteriorly directed cilium and one shiny body in the protoplast, slightly more than 2 μ thick. Spores discharged within a host cell on April 27 were still capable of motion the following day. Spores swimming as typical for uniciliate chytrid spores. Resting bodies spherical with less conspicuous spines than on the sporangia, about 12.5 thick, with a thick brownish inner wall. The resting bodies seem to cause no distortion in the threads.

Collected twice at Chapel Hill, N. C. In *Zygogonium* sp. Jan. 1933. J. N. Couch, coll. In *Mougeotia* sp. April 1933. Willard Hewitt, coll. This species is widespread in Europe, having been first described by Dangeard (**1889, 1890–91**) in France, in *Zygogonium;* by Petersen (**1910**) in North Sealand and Jutland, in *Mougeotia* sp.; by Denis (**1926**) in the high Pyrenees, elevation 2000 m., France, in *Spirogyra quadrata;* by Huber-Pestalozzi (**1931**) in Switzerland, in *Mougeotia* sp.; and by Heidt (**1937**) in Germany, in *Mougeotia scalaris* Hass.

UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

LITERATURE CITED

Couch, John N. *Micromyces Zygogonii* Dang., parasitic on *Spirogyra.* Jour. Elisha Mitchell Sci. Soc. **46**: 231–238. *pl. 16–18.* 1931.

Dangeard, P. A. Memoire sur les chytridinees. Le Botaniste **1**: 52–56. *pl. 2, fig. 1–10.* 1888.

——. Maladies des Algues et des Animaux. Le Botaniste **2**: 245–246. *pl. 17, fig. 3–8.* 1891.

Denis, M. La Castration des Spirogyres par des Champignons parasites. Revue Algologique **3**: 14–21. *fig. 2.* 1926.

Heidt, Karl. Beitrag zur Kenntnis der Gattung micromyces. Ber. Deuts. Bot. Ges. **55**: 204–217. *fig. 1–8.* 1937.

Huber-Pestalozzi, G. Infektion einer *Mougeotia*—Population durch *Micromyces zygogonii* Dang. an einem alpinen Standort. Hedwigia **71**: 88. 1931.

Petersen, H. E. An account of Danish Freshwater Phycomycetes. Ann. Myc. **8**: 556. *fig. 27c.* 1910.

Scherffel, A. Einiges über neue oder ungenügend bekannte Chytridineen. Archiv Protistenkunde **54**: 167–260. *pl. 9–11.* 1926.

EXPLANATION OF FIGURE

All figures except 13 drawn under Leitz 1.4 n. A. oil imm objective and 8 ocular, giving a magnification of 1250. Fig. 13. 2400. All figures reduced one-half in reproduction.

Fig. 1–8. *Micromyces Zygogonii* Dangeard. 1, zoöspores on *Mougeotia* thread, ready to penetrate. 2, host cell containing parasite (par). Host protoplast shows considerable collapse; host nucleus (h.n.) is in contact with parasite. Nucleus of parasite is distinct, as are also the young spines. 3, mature prosorus with distinct nucleus and spines. Host cell is swollen and bent and of the host protoplast there remains only a disorganized mass of greenish matter and numerous granules perhaps starch. 4, host cell has burst. Prosorus has discharged to form sorus, the contents of which have begun to cleave into sporangia. 5, empty prosori with sori, each composed of four sporangia. 6, empty prosorus with old host nucleus (h.n.) attached. Sorus has divided into four sporangia. 7, sorus with four sporangia three of which have discharged spores (sp.), two of which are apparently fusing. 8, sorus of four sporangia all of which have discharged spores (sp.). After 24 hours some of these spores were still capable of swimming. Note host nucleus (h.n.). 9–14. *Micromyces longispinosus.* These figures are taken from Couch (**1931**). Due to limitations of space, the host cell walls are shown only in fig. 12. 9, sorus showing long spines, host nucleus (h.n.) and parasite nucleus. 10, empty prosorus and sorus. Note granules, perhaps nuclei, in sorus. 11, sorus divided into sporangia. 12, sorus of sporangia broken up. Some sporangia are discharging spores, others are empty. Some of the spores are apparently fusing. 13, single sporangium discharging spores some of which are apparently fusing. 14, resting body in process of germination; note vesicle from which protoplasm has partly collapsed, dis. ch., disintegrated chloroplast; h.n., host nucleus.

THE OCCURRENCE OF A HUMAN PATHOGENIC FUNGUS AS A SAPROPHYTE IN NATURE

NORMAN F. CONANT

The feeling that many of the fungi pathogenic for man must occur as saprophytes or parasites in some unrecognized natural habitat has existed for several years. Attempts to link the skin fungi or dermatophytes with forms already known to occur in nature on substrates of horny material have, as yet, produced no complete evidence of identity. Likewise, several attempts to isolate skin fungi from shower bath floors, swimming pools, and other likely places of contamination have failed to produce forms known to be pathogenic for man.

One fungus, however, *Phialophora verrucosa* Thaxter in Medlar 1915, a form which has been isolated from cases of chromoblastomycosis in Boston, Texas, Uruguay, and other South American localities can now be definitely identified with fungi of the genus *Cadophora* which have been isolated from pulp wood and unfinished pine and spruce timbers in this country and Europe.

When Thaxter described the endogenous manner of conidial formation of *Phialophora verrucosa* in 1915, he suggested that "the fungus should be classed under the sub-division Chalareae of Saccardo's classification and should be the type species of a new genus." Likewise, Lagerberg and Melin when describing the conidial formation of *Cadophora fastigiata* in 1927 suggested that the fungus be placed in the Chalareae of the Dematiaceae. In 1934 five other species were added to the genus *Cadophora* by Melin and Nannfeldt, while in 1935 Davidson added two more.

A careful comparative study of the eight species of *Cadophora* described from lumber and pulp wood and three strains of *Phialophora verrucosa* isolated from cases of chromoblastomycosis has shown that these fungi belong to the same genus. While different species exist, the generic character of endogenous conidial

formation from ampullaceous conidiophores bearing funnel or cup-like structures at the apices, is common to all of the fungi. The genus *Cadophora* Lagerberg and Melin 1927 must, therefore, fall into synonomy with *Phialophora* Thaxter 1915. Therefore the following new synonomy and new combinations are made; thus, *Cadophora americana* Nannf. becomes a synonym of *Phialophora verrucosa* Thaxter, and the following species of *Cadophora* are transferred: **Phialophora fastigiata** (Lagerb. & Melin) Conant, **Phialophora brunnescens** (Davidson) Conant, **Phialophora Lagerbergii** (Melin & Nannf.) Conant, **Phialophora Meliniï** (Nannf.) Conant, **Phialophora obscura** (Nannf.) Conant, **Phialophora repens** (Davidson) Conant, and **Phialophora Richardsiae** (Nannf.) Conant.

A full report of the work concerning these fungi will appear later.

DEPARTMENT OF BACTERIOLOGY AND MEDICINE,
DUKE HOSPITAL,
DURHAM, NORTH CAROLINA

LITERATURE CITED

1. **Medlar, E. M.** A new fungus, *Phialpohora verrucosa,* pathogenic for man. Mycologia **7**: 200–203. 1915.
2. **Lagerberg, T., Lundberg, G. & Melin, E.** Biological and practical researches into blueing in pine and spruce. Svenska Skogsvards. Tidskr. **25**: 145–272. 1927.
3. **Melin,.E. & Nannfeldt, J. A.** Researches into the blueing of ground wood-pulp. Svenska Skogsvards. Tidskr. **32**: 397–585. 1934.
4. **Davidson, R. W.** Fungi causing stain in logs and lumber in the southern states including five new species. Jour. Agr. Res. **50**: 789–807. 1935.

STUDIES OF CERTAIN SPECIES OF MELANCONIS ON CARPINUS, OSTRYA AND CORYLUS [1]

LEWIS E. WEHMEYER

(WITH 3 FIGURES)

There occur, upon *Carpinus, Ostrya* and *Corylus,* a difficult group of related species of *Melanconis* which have been described under many names in a number of genera. Although this group of species, or varieties, shows a rather wide range of variation in certain characters, it is difficult to distinguish morphologic species because of the overlapping ranges of variation. The literature and exsiccati of these species are also extremely confusing. It is the purpose of this paper to present and review certain data which may help to clear up this situation.

PERITHECIAL STAGE

A study of a large number of collections and exsiccati of this group of species from various hosts, from both Europe and America, and both in the fresh and dried condition, shows that there is a great deal of variation in a number of characters. Superficially, they vary from minute pustules to rather widely erumpent discs. The ectostroma varies in size from 250–2000 μ or more in diameter and from a dull gray, through gray-green to yellow- or olive-green in color, and may be sharply outlined or merged with a slight entostromatic development in the bark. The spores, when young, are small fusoid with tapered ends which may bear faint pointed or globose evanescent appendages. With maturity, they become rounded at the ends and constricted at the septum. In dried material, the protoplast may shrink giving the appearance of a thickened wall. In size, the spores are from 11–27 by 3–9.5 μ. The asci vary from 50–127 $\times$ 6.5–14 μ. In spite of these variations,

[1] Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 621.

very little correlation could be found between the different characters or between these and the host.

In a previous study (**14**), this group was broken up into three species (*M. xanthostroma, M. hyperopta* and *M. sulphurea*). A tabulation by hosts, of spore measurements from some forty-one European and twenty-four American collections indicated that there were two rather than three species represented. One of these, *M. sulphurea,* on *Corylus,* has larger spores (18) 20–27 × 6–9.5 μ, whereas the second, *M. xanthostroma,* on *Carpinus* and *Ostrya,* has somewhat smaller spores (10) 11.5–20 (23) × (3) 3.5–5 (7) μ. The spores found on *Carpinus* and *Ostrya* show two general groups, one measuring 11–15 × 3–5 μ and the other 15–23 × 5–6 μ, but there are many collections showing overlapping ranges as regards spore size. That this difference in spore size is due, in part at least, to the maturity of the spore and the vigor of development, is supported by the fact that one collection on *Carpinus* (Wehm. Herb. No. 3625) which had spores measuring 11.5–15 × 3–4 μ when collected, yielded spores of this size and also larger ones measuring 17–20 × 5–6.5 μ, after four to five days in a damp chamber. It is also true that the European collections on *Carpinus* show a slightly narrower range of spore size (15–20 (23) × 3.5–7 μ). Cultural data, which will be presented later, also show that there is in America a distinct species on *Ostrya.*

On the information available, and until further cultural data can be obtained, it seems best to arrange the various synonyms under three species occurring upon the three genera as follows:

MELANCONIS XANTHOSTROMA (Mont.) Schroet. Krypt.-Fl. Schles. **2**: 441. 1897.

Sphaeria xanthostroma Mont. Ann. Sci. Nat. II. **1**: 301. 1834.

Valsa chrysostroma Fries, Sum. Veg. Scand. 412. 1846.

Valsa xanthostroma (Mont.) Tul. Ann. Sci. Nat. IV. **5**: 117. 1856.

Valsa bitorulosa Berk. & Br. Ann. & Mag. Nat. Hist. III. **3**: 367. 1859.

Valsaria bitorulosa (Berk. & Br.) de Not. in Rab. Fung. Eur. 932.

Melanconis chrysostroma (Fries) Tul. Fung. Carp. **2**: 125. 1863.
Diaporthe hyperopta Nit. in Otth, Nachtr. 6, Mittheil. nat. Ges. Bern, 1868: 47.
Diaporthe carpinicola Fuckel, Symb. Myc. Nachtr. **2**: 37. 1873.
Diaporthe decipiens Sacc. Fung. Ven. **4**: 6. 1875.
Diaporthe nigro-annulata Kunze, in Kunze. Fung. Sel. 122. 1875.
Sphaeria carpinigera Berk. & Curt. in Curt. Herb.
Diatrype carpinigera Berk. & Curt. Grevillea **4**: 96. 1876.
Diaporthe Kunzeana Sacc. Fung. Ven. **5**: 180. 1876.
Cryptospora bitorulosa (Berk. & Br.) Niessl, Hedwigia **16**: 119. 1877.
Cryptospora nigro-annulata (Kunze) Rehm in Thüm. Myc. Univ. *2063*. 1881.
Diaporthe bitorulosa (Berk. & Br.) Sacc. Syll. Fung. **1**: 608. 1882.
Diaporthe Carpini Sacc. nec Fuckel, Syll. Fung. **1**: 607. 1882 (Myc. Ven. Spec. 137).
Diaporthe mucosa Wint. in Sacc. Syll. Fung. **1**: 609. 1882.
Diaporthe carpinigera (Berk. & C.) Sacc. Syll. Fung. **2**, Add. XLVIII. 1883.
Diaporthe Ellisii Rehm, Bull. Torrey Club **10**: 89. 1883.
Valsa Ellisii Rehm in Ellis & Ev. N. Am. Fungi *1567*. 1886.
Diaporthe farinosa Peck, N. Y. State Mus. Rep. **40**: 69. 1887.
Melanoconis bitorulosa (Berk. & Br.) Ellis & Ev. North Am. Pyrenom. 522. 1892.
Diaporthe leucostoma Peltg. in Höhnel, Sitz. Acad. Wiss. Wien **115**: 1251. 1900.
Chorostate decipiens (Sacc.) Trav. Fl. Ital. Crypt. **2**: 208. 1906.
Chorostate Kunzeana (Sacc.) Trav. Fl. Ital. Crypt. **2**: 200. 1906.
Chorostate mucosa (Wint.) Trav. Fl. Ital. Crypt. **2**: 192. 1906.
Chorostate suspecta Sacc. Ann. Myc. **11**: 115. 1913.
Discodiaporthe xanthostroma (Mont.) Petr. Hedwigia **62**: 293. 1920.

Melanconis Ostryae (Dearn.) comb. nov.

Diaporthe ostryigena Ellis & Ev. in Herb. (N. Am. Fungi *3420*) 1896.
Diaporthe Ostryae Dearn. Mycologia **18**: 246. 1926.

Melanconis flavo-virens (Otth.) comb. nov.

Valsa flavo-virens Otth. Nachtr. 6, Mittheil. Nat. Ges. Bern. 1868: 47.
Diaporthe sulphurea Fuckel, Symb. Myc. 205. 1869.
Diaporthe affinis Sacc. Michelia **1**: 28. 1878.
Valsa olivaestroma Cooke, Grevillea **14**: 48. 1886.
Diaporthe elaeostroma (Cooke) Berl. & Vogl. Syll. Add. 107. 1886.
Chorostate sulphurea (Fuckel) Trav. Fl. Ital. Crypt. **2**: 209. 1906.
Chorostate affinis (Sacc.) Trav. Fl. Ital. Crypt. **2**: 209. 1906.
Discodiaporthe sulphurea (Fuckel) Petr. Hedwigia **62**: 291. 1921.
Melanconis sulphurea (Fuckel) Petr. Ann. Myc. **21**: 321. 1923.

CONIDIAL STAGE

A number of collections examined have shown associated conidial stages. Table 1 will serve as a reference for these associations.

From this table it can be seen that three types of conidia have been found associated with this species. First, a small cylindric to allantoid, one-celled, hyaline form measuring (5.5) 8–14 × 1.5–2.5 μ. This type was found on both European and American material, but always on Carpinus.[2] Second, a larger, fusoid to clavate, one-celled, hyaline type measuring 17–27.5 × 4–5 μ and found only on American material on *Carpinus*. Third, a *Melanconium* type of pustule with ovoid, brown, one-celled conidia, 11–16 × 5–10 μ occurring on *Ostrya* in America and on *Carpinus* in Europe. *Melanconium triangulare* Ellis & Ev., although given as the conidial stage of *D. Ellisii*, is probably in error.

The associations of conidial stages with the various species of this group to be found in the literature are numerous and confusing. A brief survey of these will be considered here.

On *Carpinus:*

Melanconium bicolor beta ramulorum Corda.
Melanconium microsporum Nees.

[2] The *D. Bloxami* specimens are given as on *Fagus*, but seem to be *Melanconis xanthostroma* on *Carpinus*. These somewhat larger conidia may, however, belong to some other species.

TABLE 1

Collection	Cylindric, hyaline conidia	Fusoid, hyaline conidia	Ovoid, brown conidia
Syd. Myc. March. *263*, sub *Cryptospora bitorulosa* (C)	8–10×2.5		
Syd. Myc. March. *2947*, sub *Diaporthe Bloxami* (F?)	11–14×2–2.5		
Rehm Asc. *980*, sub *Diaporthe Bloxami* (F?)	11–14×2–2.5		
Thüm. Myc. Univ. *469*, sub *Diaporthe decipiens* (C)	8–11×2–2.5		
Ellis N. A. F. *1567*, sub *Diaporthe Ellisii* (C)	8–11×2		
Wehm. Herb. *3647*, sub *Melanconis hyperopta* (C)	5.5–8.3×2–2.5		
Wehm. Herb. *3645*, sub *Melanconis hyperopta* (C)	10–12×1.5–2		
Wehm. Herb. *3625*, sub *Melanconis hyperopta* (C)	8–10×2.5–3	17–23.5×4–5	
Rab. Fung. Eur. *2243*, sub *Cryptospora bitorulosa* (C)	9–12×2–2.5		
Wehm. Herb. *3624*, sub *Melanconis hyperopta* (C)		18–27×4–5	
Rab. Fung. Eur. *2243b*, sub *Cryptospora bitorulosa* (C)			11–14×7–10
Syd. Myc. March. *984*, sub *Diaporthe decipiens* (C)			10.5–16×8–9
Dearness Herb., July 6, 1904, sub *Diaporthe Ostryae* (O)			11–15×5–7
Wehm. Herb. *3828*, sub *Melanconis Ostryae* (O)			10–12×5–6.5
Wehm. Herb. *3829*, sub *Melanconis Ostryae*(O)			11–13×5–6.5
Ellis N. A. F. *1568*, sub *Melanconium triangulare* (C)			5–6 μ diam.

The host is represented by the capital initial, as *Carpinus* (C), *Ostrya* (O), *Fagus* (F).

This conidial stage was given by the Tulasnes (**13**, p. 125) as belonging to *Melanconis chrysostroma*. They state that on the surface of a covered ectostroma there are formed two types of conidia. One, *Melanconium*-like with ovate dark colored conidia, acute at one end and measuring 13–15 × 10 μ, and a second "Cytisporoid" type with ovate to linear oblong conidia, sometimes curved, hyaline, and 10–13 × 3.5–5 μ.

Fuckel (**5**, p. 37) gives the spermagonia of his *Diaporthe carpinicola* as unilocular, tuberculiform, beneath the epidermis and with conidia which are elongate fusiform, one-septate, 2–3 guttulate, straight, hyaline and 10–12 × 5 μ. The septation seems doubtful.

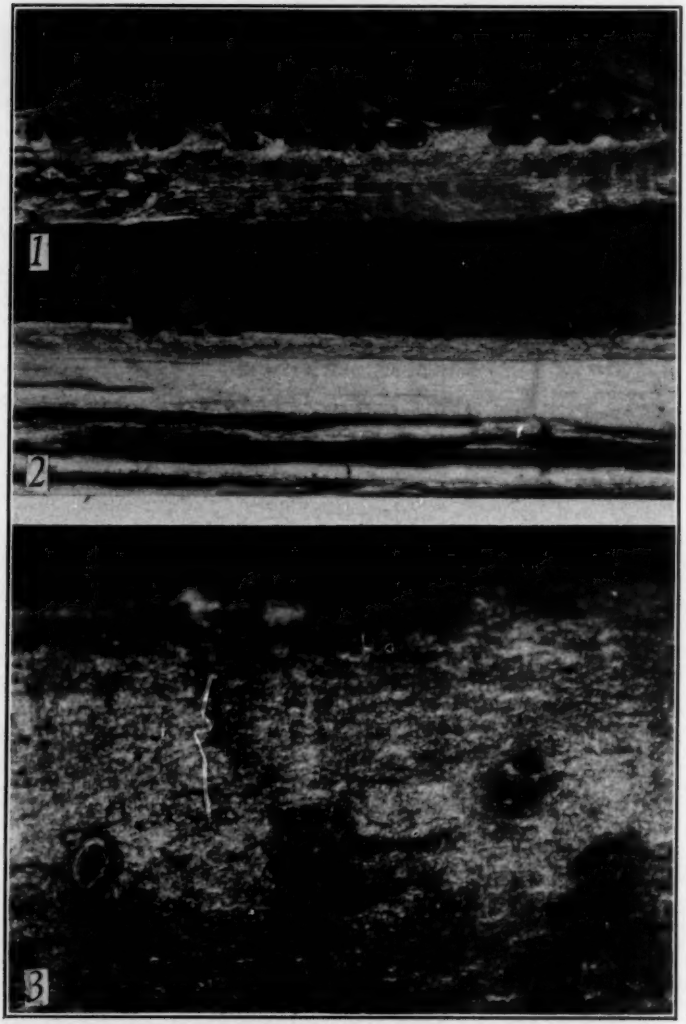


FIG. 1. *Melanconis* sp.

In Rab. Fungi Eur. *2243,* Niessl gives out material containing hyaline conidia, which he gives (**12**, p. 119) as measuring 11–14 × 4 μ and as belonging to *Cryptospora bitorulosa* (*Diaporthe Kunzeana*). The writer finds these to be the typical cylindric hyaline conidia and measuring 9–12 × 2–2.5 μ. In No. *2243b* of this same exsiccati, he issues a *Melanconium* type of conidial stage which he says is connected with *Melanconis chrysostroma,* which in turn differs from his *Cryptospora* in the greenish-yellow stroma. Both these conidial stages agree with the Tulasne's descriptions of *M. chrysostroma.*

Cytispora sp. was cited by Ellis (**2**, p. 89) as the conidial association for *Diaporthe Ellisii.* The oblong, hyaline, 4-nucleate conidia, measuring 8–11 × 2–2.5 μ and occurring in orange colored masses, which he describes, are typical of the smaller conidial type on *Carpinus.*

Melanconium triangulare Ellis & Ev., which was later suggested by Ellis (**3**, p. 38) as a conidial stage of *D. Ellisii* undoubtedly belongs elsewhere for the triangular conidia are not similar to any of the conidial stages associated with this group.

Fusicoccum Kunzeanum was given by Saccardo (Syll. Fung. **1**: 607) as the conidial stage of *Diaporthe Kunzeana* (*D. Carpini* Sacc. nec. Fuckel). It is described as having a depressed conic stroma beneath the periderm with a single locule and with oblong-fusoid, straight or curved conidia, measuring 10–11 × 3–4 μ. These again seem to be the small cylindric conidial type.

In 1906, Höhnel (**6**, p. 681) listed *Fusicoccum Carpini* as the conidial stage of *Diaporthe Carpini* Sacc. This is an error for *F. Carpini* was described as the conidial stage of *D. Carpini* Fuckel nec Saccardo, which is an entirely different species.

Melanconium deplanatum Speg.

Myxosporium deplanatum (Lib.) Sacc.

Discosporium deplanatum (Lib.) Höhn.

Höhnel (**7**, p. 197) in his discussion of his new genus *Discosporium,* gives the above synonomy and points out that this species is the same as the conidial stage described by Fuckel for his *Diaporthe carpinicola.* He claims that there are perithecial stromata of *Melanconis chrysostroma* on Fuckel's exsiccati and that this conidial stage belongs to that species and not to *D.*

carpinicola, not recognizing the identity of the two perithecial stages. He also points out the identity of the conidia of this species with the "cytisporoid" conidia of the Tulasne's description and says that he has seen hyaline ovoid conidae, 14 × 10 μ in these locules, which he considers to be immature Malanconium conidia.

Malacostroma irregulare (Died.) Höhn.

Cytospora carnea Ellis & Ev.

Dothiorella irregularis Died.

Höhnel (**8**, p. 355) gives the above synonymy for the conidial stage of *Diaporthe farinosa* Peck. This *Diaporthe* was originally given as occurring on *Tilia*, but obviously is on *Carpinus*. These conidial stages occur on *Tilia*, have large tuberculate, multilocular stromata and conidia which are elongate fusoid, two-guttulate and 8–12 × 2.5–3 μ. This is undoubtedly an incorrect guess.

On *Corylus:*

Myxosporium sulphureum Sacc.

This is given as the conidial stage of *Diaporthe sulphurea* Fuckel by Saccardo (Syll. Fung. 1: 625). The conidia are given as fusiform, inaequilateral, one-celled and 12–16 × 5–6 μ. These conidia seem to be of the fusiform hyaline type found on *Carpinus* but are shorter than those obtained from American material on that host.

Discosporium sulphureum (Sacc.) Petr.

Petrak (**10**, p. 291) has pointed out the similarity between *Myxosporium sulphureum* Sacc. and *Discosporium deplanatum* (Lib.) Höhn, and places *M. sulphureum* in the genus *Discosporium*. Petrak is of the opinion that *Melanconium bicolor beta ramulorum*, occasionally found on *Carpinus*, has no connection with *M. xanthostroma*. He considers the conidial stages of both *M. xanthostroma* and *M. sulphurea* as a *Phomopsis* with an open cavity (*Discosporium*) and erects a new genus, *Discodiaporthe*, to accommodate these two species. In a later paper (**11**, p. 321) he concludes that this conidial stage is in reality a hyaline spored *Melanconium* and that the difference in the conidial stage between *Melanconis* and his *Discodiaporthe* is not of generic importance and therefore unites these two genera.

These reports in the literature seem to indicate that there is a

species on *Carpinus* with a *Melanconium* type of conidial stage with a dark brown, ovoid, one-celled, and a cylindric, hyaline, one-celled type of conidium. The reports of conidial stages on *Corylus* are more sparse but indicate one type of conidium which is fusoid, inaequilateral, hyaline and one-celled. A further consideration of these conidial stages and their relation to the American species will be postponed until after a presentation of the cultural data.

CULTURAL RESULTS

Melanconis xanthostroma (Mont.) Schroet., which name may be used tentatively for the American species on *Carpinus,* was the first to be cultured, from material collected near Ann Arbor, Michigan, in November, 1935. Sprays of ascospores were made onto nutrient agar on November 12 and again on December 7, 1935. No germination was evident in either case until after forty-eight hours, when a few spores showed short germ tubes. The spores became irregularly swollen (FIG. 2: 4) and measured 18–20 × 5.5–6.5 μ. They put out from one to three germ tubes 3–3.5 μ in diameter, from one or both cells. Growth from isolated ascospores was exceedingly slow on nutrient agar, but much more rapid on oat agar where colonies 1.5–2 cm. in diameter were produced in about two weeks. A rather abundant compacted cottony white mycelium was formed on the surface. The growth became yellow-green or gray-green with age. In old cultures, irregular pulvinate masses of compacted hyphae were formed on the surface, from which were exuded pinkish spore masses. The spores in these droplets were fusoid, one-celled, hyaline, often abnormally curved or bent and measured 16–25 × 3.5–5 μ.

On May 2, 1936, autoclaved twigs of *Carpinus caroliniana* were inoculated from single ascospore cultures. Growth was very slow on twigs also appearing superficially, only after a month's incubation, as a whitish to yellowish cottony mycelium at the point of inoculation. After about six weeks, yellow-orange spore horns appeared at these points. Afterwards, more normal conidial pustules (FIG. 2: 1) were formed more rapidly over the entire surface. The ectostromata originate on the bark surface beneath the periderm and consist of a yellowish green prosenchyma. The

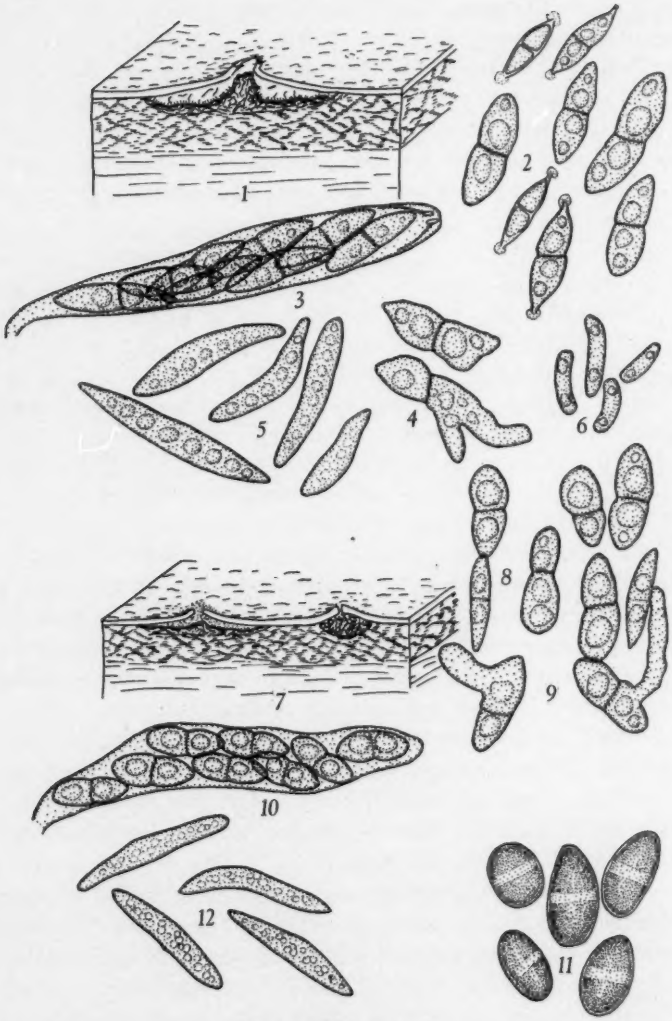


FIG. 2. *Melanconis* sp.

central portion grows upward and ruptures the periderm and becomes the erumpent disc which is olive-green in color. The basal portions spread out centrifugally, and on these flanks or the sur-

face of this portion, around the central sterile disc, the hymenium is formed. As a result the spore masses are pushed out at the sides of the central disc, not through it. The conidia (FIG. 2: 5) are variable in size and shape, especially so in old pustules or under unfavorable growth conditions. They are cylindric-fusoid to ellipsoid-fusoid, straight to curved or bent, one-celled, hyaline, show a single row of conspicuous droplets when fresh and measure from 16.5–30 (37) × 3.5–5 μ. The spore masses are either pinkish or yellow at first, becoming dark olive brown when dry. These alpha conidia, as they appear to be, correspond to the fusoid hyaline type found twice in nature. Scores of pustules of all ages were examined in a search for the beta or cylindric type of conidium (FIG. 2: 6), commonly found associated with this species in nature, but none were found. In old pustules the alpha conidia may become narrower or shorter and approach the beta type in size, but these should not be considered as such. It is probable that the rather vigorous growth under the moisture conditions of the culture tubes is not favorable for the formation of the cylindric conidia, for in spite of their absence from culture, the writer is still of the opinion that they represent a second conidial type for this species.

Similar inoculations onto twigs of *Ostrya virginiana* made on July 3, 1936, gave very similar results. The conidial stromata tended to be somewhat smaller and the amount of spore production was greatly reduced but the stromata and conidia were of the same type, the latter measuring 15–23.5 × 3.5–5.5 μ.

Melanconis Ostryae (Dearn.) comb. nov.

The lack of any dark walled conidia in the life history of the species on *Carpinus caroliniana* and the occurrence of a *Melanconium* stage on type material of *Diaporthe Ostryae* from Dr. Dearness, suggested a closer consideration of this species on *Ostrya*. Several occurrences of a *Melanconium* in association with *Melanconis* (*Diaporthe*) *Ostryae* were soon found in the field. Three sets of spray cultures on nutrient agar were made, and single spores isolated from this material as follows:

3828. S. Ascospores from perithecia on *Ostrya virginiana* collected July 29, 1936, near Ann Arbor, Mich.

3828. C. *Melanconium* conidia from pustules on the same twigs as 3828 S.

3829. Ascospores from perithecia on *Ostrya virginiana* collected August 6, 1936, near South Lyons, Mich.

The ascospores (FIG. 2: 8) of *M. Ostryae* germinated within twenty-four hours by means of a single germ tube (FIG. 2: 9) some 3–4 μ in diameter. The germinating spores measured 15–18 $\times$ 5.5–6.5 μ. Growth, in this species, was also slow but much more rapid than in *M. xanthostroma*. On nutrient agar the growth was slow (about 4 cm. in five weeks) and a distinct circular colony was formed with a wrinkled marginal zone of appressed pubescent, white mycelium. The center of the colony turned gray-brown to tan with age, but did not show the yellowish or yellow-green tints of *M. xanthostroma*. On oat agar the growth was more rapid and irregular and stromata were formed sooner and in greater abundance. Blackish spore masses were exuded from these stromata. These conidia (FIG. 2: 11) were ovoid to ovoid-oblong, one-celled, olive-brown and measured 10.5–14 $\times$ 6–7 μ. These growth characters and the conidial formation were practically identical in each isolation (*3828 S* and *3829*). In a few spore horns from young stromata of 3828 on oat agar, a second, or beta type of conidium was found. These conidia (FIG. 2: 12) were elongate-fusoid, one-celled, hyaline, irregularly granular and 19.5–25 $\times$ 2.5–3.5 μ. These conidia, which are similar to the alpha conidia of *M. xanthostroma* were rare, or entirely absent from the older spore horns and were never seen again in culture. This scarcity in culture may explain the failure to find beta conidia of *M. xanthostroma*.

The *Melanconium* conidia from *Ostrya* (*3828 C*) germinated within twenty-four hours by means of a single germ tube, 3–3.5 μ in diameter and gave rise to a colony similar in all respects to the ascospore isolations. The same type of stromata and the same *Melanconium* type of conidium were also obtained from these cultures.

Inoculations were made onto twigs of *Ostrya virginiana* on October 7, 1936, and onto *Carpinus caroliniana* on November 10 from all three of the isolations mentioned. The results were es-

sentially the same in all cases. Growth and fruiting occurred equally as well on *Carpinus* as on *Ostrya*. Superficial, spherical, white to grayish stromata which exuded watery black spore masses were first formed in the moist tubes. The more normal ectostromata (FIG. 2:7) were formed later on the surface of the bark cortex just beneath the periderm. A conidial hymenium is formed first on the flanks and eventually over the entire surface of this gray-green ectostroma which is usually filled with crystalline granules. At maturity there remains a very thin layer of light colored stroma with the hymenial layer. The mass of spores formed are pushed out through a pore like opening in the periderm as a coiled thread or ribbon of spores. The conidia (FIG. 2: 11) are oblong-ellipsoid to ovoid, one-celled, hyaline at first, but soon olive-brown, coarsely granular and usually with a light colored equatorial band about the cell. They measure 8.5–14 × 6–7.5 μ.

In some cultures after the formation of conidial ectostromata, and usually on other portions of the twigs, there arose small spheric to conic ectostromata 150–250 μ in diameter. These occasionally had slight shallow cavities with a few spores on their flanks, but were normally sterile and undoubtedly represent the initiation of perithecial pustules.

This production of a *Melanconium* conidial stage by the form on *Ostrya* indicates that there is a distinct species on this host. A careful study of what material was available on *Carpinus* and Ostrya indicates certain other correlated characters which may be outlined as follows:

M. xanthostroma on *Carpinus* has larger (500–1200 μ diam.) more strongly erumpent ectostromata (FIG. 1:1) which often take on a more yellow-green color. The discs are generally larger (300–800 μ) and more elongate. The spores are more variable in size; immature spores being 11–15 × 3–5 μ, whereas fully mature spores reach 15–23 × 4–7 μ. Growth in culture is very slow and shows yellow-green to olive shades. No brown conidia formed.

M. Ostryae on *Ostrya* has minute pustules with smaller (200–600 μ) less strongly erumpent ectostromata (FIG. 1: 2) which are more gray-green in color. The discs are smaller (200–400 μ)

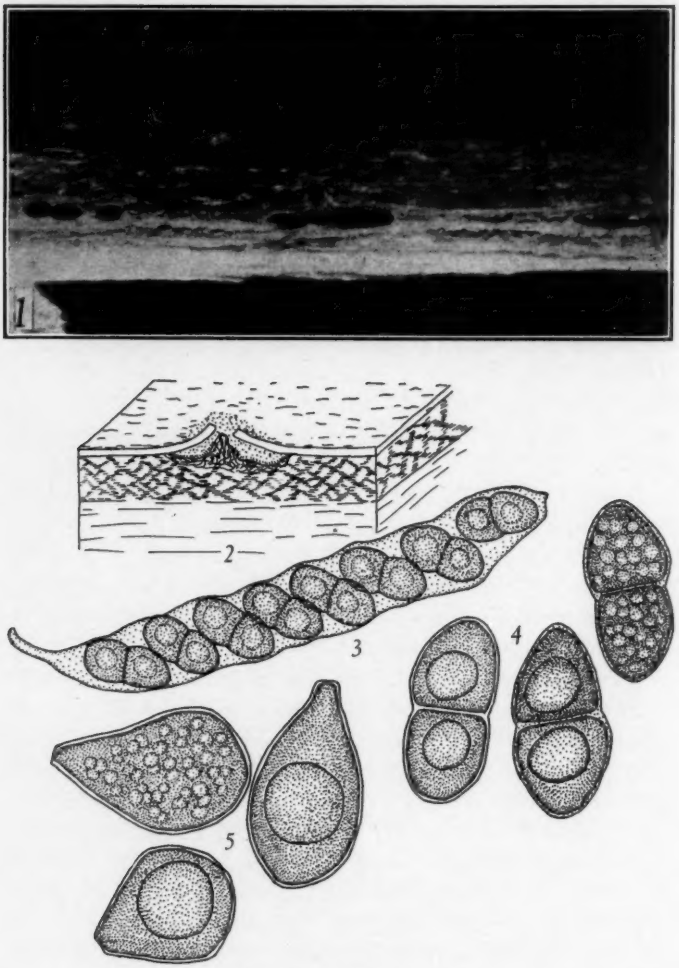


FIG. 3. *Melanconis* sp.

and more circular. The spores are not so variable, measuring 14–21 × 4–7 μ, and often have one cell narrower and somewhat tapered. Growth in culture shows gray-brown to tan shades. Brown ovoid conidia are formed.

While collecting material for these studies, a *Melanconium* was also found on *Carpinus*, near Brighton, Michigan. Upon examination, this was found to be associated with a *Melanconis* with brown spores which has proved to be a new species, and is described here as follows:

Melanconis platystroma sp. nov.[3] (FIG. 1: 3, FIG. 3: 1–5).

Appearing on the surface (FIG. 1: 3) as flattened circular pustules with a central conical papilla through which there is finally erumpent a truncate conic, gray to yellow-gray ectostromatic disc, 0.5–0.8 mm. in diameter. The cylindric ostioles are scarcely visible or barely erumpent through the disc. The perithecia are 400–500 μ in diameter, somewhat flattened, circinate beneath the ectostroma (FIG. 3: 1) in the upper bark cortex, which is unaltered or slightly entostromatic. The asci (FIG. 3: 3) are cylindric, pinched off at the base, about 200 $\times$ 14–17 μ. The spores (FIG. 3: 4) are uniseriate or overlapping, blunt ellipsoid, two-celled, constricted and occasionally slightly bent at the septum, brown to olive-brown, coarsely granular when fresh, becoming uniguttulate, and 25–31 $\times$ 12.5–14.5 μ. The spore wall is brown, but the fresh spore has a greenish tint due to the color of the granular content. The paraphyses are broad band-like but soon evanescent.

[3] *Melanconis platystroma* sp. nov. Stromata in superficie comparantia sicut pustulae rotundae planae papilla conica centrali per quam discus ectostromaticus conicus truncatus canus vel luteo-canus 0.5–0.8 mm. diametro demum erumpit. Ostiola cylindrica haud facile manifesta vel vix per discum erumpentes. Perithecia sub ectostromate in cortici superficiali circinata, 400–500 μ diametro. Asci cylindrici, basi praemorsi, 200 $\times$ 14–17 μ. Sporae uniseriatae vel imbricatae, obtuso-ellipsoideae, bicellulae, in septo constrictae et interdum leviter curvatae, fuscae vel oliveo-fuscae, 25–31 $\times$ 12.5–14.5 μ; sporae vivae crasse granulares demum uniguttulatae. Paraphyses ligamentis latis, similes mox evanescentes.

Stromata conidialia superficialia Melanconii pustulis similia cumulis sporarum nigris. Hymenium conidiale in lateribus ectostromatis conici pallide vel oliveo-fusci sub periderma. Conidia sicut cumuli sporarum nigri exsudantia, angulato-globosa vel ovoidea vel pyriformia, unicellula, viridi-fusca et primo crasse granulata deinde fusca uniguttulataque, apiculata, 20–31 $\times$ 16–18 μ, in conidiophoribus brevibus.

In ramis *Carpini carolinianae* in sylvis "Pleasant Valley" dictis, Brighton, Michigan, 21 October, 1936. Specimina typica in Herbb. Univ. Mich. et auctoris.

Conidial stage: On surface as *Melanconium*-like pustules with black masses of spores. Conidial cavities (FIG. 3: 2) borne on the flanks of a conic, pale to olive-brown ectostroma beneath the periderm. Conidia (FIG. 3: 5) exuded as black spore masses, angular-globose to ovoid or pyriform, one-celled, greenish-brown and coarsely granular at first, becoming brown and uniguttulate, apiculate, borne on short conidophores and measuring 20–31 × 16–18 μ.

On branches of *Carpinus caroliniana,* Pleasant Valley Woods, Brighton, Mich., October 21, 1936. Type specimens deposited in University of Michigan Herbarium and in author's Herbarium.

Numerous attempts have been made to germinate both the ascospores and conidia of this species, but so far all of these have failed. Twigs with both perithecial and conidial pustules were matured in damp chambers and healthy growing spores of all ages of maturity were obtained. Suspensions consisting of a mixture of conidia and ascospores in sterile distilled water were treated in various ways and then sprayed onto agar plates, as follows, in order to induce germination:

1. Suspensions and plates placed at 40° and 60° C. for 24 hrs. and then removed to room temperature.
2. Suspensions and plates placed for twenty-four hours at 2° and 10° C. alternately with similar periods at room temperature.
3. Suspensions made in dilute solutions of KOH (0.5 per cent) and lactic acid (0.5 and 2.5 per cent) before spraying onto plates.
4. Suspensions made in a dilute infusion of *Carpinus* bark and sprayed onto plates.
5. Suspensions made in several high dilutions of Indol-acetic acid.

Both the suspensions and the plates were kept under observation for 10–20 days, when contaminations overran the plates, but not a single germinating ascospore or conidium was seen. A few spores on the lactic acid plates showed an exudation of hyaline droplets but no further development occurred. Suspensions were also

sprayed onto autoclaved twigs of *Carpinus* but no infection has been observed.

During the period that these twigs were in the damp chamber, perithecial pustules of *M. xanthostroma* also appeared. The *Melanconium* pustules were always associated with *Melanconis platystroma* perithecial pustules, however, and apparently belong to this species.

DISCUSSION

From the foregoing discussion we see that a tabulation of spore size suggests two species in this group, one on *Carpinus* and *Ostrya* and one on *Corylus*. Cultural studies of the American species show that there are two similar but distinct species, one on *Carpinus* and one on *Ostrya*, differing in the conidial stages. Only cultural studies of the European forms and of material from *Corylus* can definitely determine the final status of species in this group, but certain suggestions do arise.

In the first place, specific (or varietal) differences seem to be correlated with the host. Stromata on *Carpinus*, both from Europe and America, are very similar and both show a cylindric hyaline type of conidium associated with these stromata. In cultures of American material, on the other hand, only the fusoid hyaline type of conidium, which has been found associated with American material in the field, was obtained. The absence of the cylindric conidia may be due to the more vigorous growth in culture.

European writers, again, report a *Melanconium* stage, *M. bicolor beta ramulorum* as associated with the species on *Carpinus* and such association has been seen by the writer. It seems probable, therefore, that there is a European species or variety on *Carpinus* differing from the American one in the conidial stage and having brown *Melanconium* and hyaline cylindric conidia. These *Melanconium* conidia are very similar to those obtained in culture for *M. Ostryae*, but appear to have a somewhat greater diameter. The cylindric hyaline conidia as cited in European descriptions seem to have a greater diameter than those found in American material, but this is not borne out by actual European material.

In America a species occurs on *Ostrya* with brown *Melanconium*

conidia similar to, but with smaller diameters than, those of the European species on *Carpinus*. Its second conidial type is similar to the hyaline fusoid conidium of the American species on *Carpinus*.

No conidia from *Corylus* have been seen by the writer, but Saccardo reports such a conidial stage with fusoid conidia similar to those obtained in culture for *M. xanthostroma* and *M. Ostryae* but distinctly shorter, which suggests that cultural studies will reveal one or several distinct species or varieties on *Corylus*.

These conidial stages also suggest a reappraisal of certain generic lines. The American form of *M. xanthostroma* would not fall within the genus *Melanconis*, although it is obviously closely related to the other species, as *M. Ostryae*, which have a definite *Melanconium* stage with brown conidia. It would rather belong with certain species of *Cryptodiaporthe* with a definite ectostroma, such as *C. galericulata*, which is reported as having similar *Fusicoccum* and *Myxosporium* stages in Europe, although cultures from American material failed to give a conidial stage. Many other species of *Cryptodiaporthe* are reported to have similar conidial stages. It seems that this group at least of the genus *Melanconis* has arisen from a section of the genus *Cryptodiaporthe* by the coloration of one or the other types of conidia formed and by the increased development of the ectostroma limiting the hymenium to the flanks of this tissue. The increase in pigmentation has subsequently been extended to the ascospores and the stromatic hyphae in various species. *Melanconis marginalis* is another species with dilutely colored conidia which is very closely related to *M. Alni* which is reported with brown conidia in Europe.

LITERATURE CITED

1. **Diedecke, H.** 1915. Kryptogamen-Flora der Mark Brandenburg. 9, Pilze 7. 1–962. Leipzig.
2. **Ellis, J. B. & Everhart, B. M.** 1883. New species of Fungi. Bull. Torrey Cl. **10**: 89–90.
3. —— ——. 1886. New species of fungi from various localities. Jour. Myc. **2**: 37–42.
4. —— ——. 1893. New species of North American fungi from various localities. Proc. Acad. Nat. Sci. Phila. **1893**: 128–172.
5. **Fuckel, L.** 1873. Symbolae Mycologicae. Zweiter Nachträge. 1–99.

6. **Höhnel, F.** 1906. Fragmente zur Mykologie. II. Mitteilung. Nr. 64–91. Sitz. Akad. Wiss. Wien. **115**: 649–695.
7. ——. 1914. Beiträge zur Mykologie. Zeitschr. Garungsphys. **5**: 191–215.
8. ——. 1917. Zweite vorläufige Mitteilung mycologischer Ergebnisse (Nr. 107–200). Ber. Deut. Bot. Ges. **35**: 351–353.
9. **Montagne, C.** 1834. Notice sur les Plantes Cryptogames récemment découvertes en France. Ann. Sci. Nat. II. **1**: 295–307.
10. **Petrak, F.** 1921. Mykologische Beiträge. I. Hedwigia **62**: 282–319.
11. ——. 1923. Mykologische Notizen. Ann. Myc. **21**: 182–335.
12. **Rabenhorst, L.** 1877. Fungi europaei exsiccati. Klotschii herbarii vivi mycologici continuatio. Edita nova. Series secunda. Cent. 3 (resp. 23). Hedwigia **16**: 117–126.
13. **Tulasne, L. R. & C.** 1863. Selecta Fungorum Carpologia. **2**: 1–319. Paris.
14. **Wehmeyer, L. E.** 1933. The genus *Diaporthe* and its segregates. Univ. of Mich. Studies. Sci. Ser. **9**: 1–349.

EXPLANATION OF FIGURES

FIG. 1. 1, radial section of perithecial stromata of *Melanconis xanthostroma* (Mont.) Schroet. on *Carpinus caroliniana;* 2, radial section of perithecial stromata of *Melanconis Ostryae* (Dearn.) on *Ostrya virginiana;* 3, surface view of perithecial stromata of *Melanconis platystroma.*

FIG. 2. 1, radial section of *Discosporium* type of conidial stage of *Melanconis xanthostroma* (Mont.) Schroet. as produced in culture; 2, ascospores of *Melanconis xanthostroma* (Mont.) Schroet. showing variation in size, shape and appendages; 3, ascus and ascospores of *Melanconis xanthostroma* (Mont.) Schroet.; 4, germinating spores of *Melanconis xanthostroma* (Mont.) Schroet.; 5, hyaline fusoid type of conidia of *Melanconis xanthostroma* (Mont.) Schroet.; 6, hyaline cylindric to allantoid type of conidia of *Melanconis xanthostroma* (Mont.) Schroet.; 7, radial section showing *Melanconium* stage and young perithecial ectostroma of *Melanconis Ostryae* (Dearn.) as produced in culture on *Ostrya virginiana;* 8, ascospores of *Melanconis Ostryae* (Dearn.); 9, germinating ascospores of *Melanconis Ostryae* (Dearn.); 10, Ascus and ascospores of *Melanconis Ostryae* (Dearn.); 11, ovoid brown type of conidia of *Melanconis Ostryae* (Dearn.); 12, fusoid hyaline type of conidia of *Melanconis Ostryae* (Dearn.).

FIG. 3. 1, radial section of perithecial stromata of *Melanconis platystroma;* 2, radial section of conidial stroma of *Melanconis platystroma* as formed on *Carpinus* in culture; 3, ascus and ascospores of *Melanconis platystroma;* 4, ascospores of *Melanconis platystroma;* 5, conidia of *Melanconis platystroma.*

NEW OR NOTEWORTHY FUNGI FROM PANAMA AND COLOMBIA. I

G. W. Martin

(with 29 figures)

Haplosporangium lignicola sp. nov.

Mycelio denso, albido demum dilute ochraceo; conidiophoris subulatis, brevibus, indivisis vel ramosis, continuis; conidiis globosis, nutantibus, tunica papillata vel rugosa, 11–15 μ diam.

Mycelium forming a dense, appressed turf, at first white, becoming pale ochraceous with age; hyphae slender, 2–3 μ in diameter, at first nonseptate, later giving rise to numerous conidiophores and becoming septate as the protoplasm is diverted into these structures, but dividing into short, thickened segments only in old cultures; conidiophores awl-shaped, simple or branched, often proliferating, variable in size but the unbranched ones mostly 40–60 μ in length and 6–8 μ in diameter at the swollen base, tapering to 1.5–2 μ at the tip; conidia globose, tuberculate, finally rugose, with dense contents and a thick hyaline wall, mostly 11–15 μ in diameter, averaging 13.5 μ.

Colombia: Dept. Magdalena, Sierra Nevada de Santa Marta, alt. 1250–1500 m. On rotten wood, collected August 11, 1935, and placed in moist chamber the following fall.

The genus *Haplosporangium* was based by Thaxter (Bot. Gaz. **58**: 362. 1914) on two species, both cultured from dung. One species, *H. bisporale,* bears two spores in many of the so-called sporangia, which are smooth-walled. It was secured from several localities. The other, *H. decipiens,* with rough-walled, undivided conidia, was found only on dung of *Selenodon* from Haiti and has apparently not been discovered since. The reproductive structures are referred to by Thaxter as sporangia and are almost certainly homologous with the sporangia of other members of the Mucorales, but since they are not divided into spores internally in either *H. decipiens* or *H. lignicola,* and not definitely so in *H.*

bisporale, in the first two species at least they are clearly conidia in the sense in which I believe the term should be used.

H. lignicola is obviously closely related to *H. decipiens,* from which species it differs in several characters. The conidia (FIG. 1) are much larger, 11–15 μ as compared with 8–10 μ, although there are a few smaller ones and a few larger, the extremes observed being 9 μ and 18 μ. The conidial wall is at first tuberculate (FIG. 2), and retains evidence of its tuberculate character even in the later, more rugose stages. The conidiophores, predominantly simple in young cultures (FIG. 3, 4, 5) betray an increasing tendency to branch as the culture becomes older (FIG. 6) and in old cultures exhibit extensive proliferation (FIG. 7, 8). The mycelium shows less tendency to become broken up into short, thickened segments than in *H. decipiens,* although in old cultures this development is not uncommon.

The conidia break away at maturity and germinate at once under suitable conditions by the production of one to several mycelial filaments which are definitely constricted where they pass through the conidial wall (FIG. 9). The fungus grows readily on all common laboratory media. Cultures have been deposited with the American Type Culture Collection, Chicago, and the Centraalbureau voor Schimmelcultures, Baarn.

DIPODASCUS ALBIDUS Lagerh.

This curious fungus was originally described from the mountains of Ecuador, where it was growing intermingled with numerous other organisms in the slime-flux exuding from a wounded bromeliad. A decade later, Juel found it in Sweden, growing in similar fashion on fallen birches. These two curiously scattered collections are the only ones reported thus far. It is, therefore, of interest to add to the record the collection from the Sierra Nevada de Santa Marta of Colombia (Hacienda Cincinnati, 1250–1500 m., August, 1935. G.W.M. *3855*). The fungus was growing on a fallen, but scarcely decayed branch which may still have had some sap in it, and had the appearance of a soft *Sebacina,* for which it was collected. Its true nature was not recognized until fourteen months later, and while attempts were made to culture it, it is scarcely surprising that these met with no success. The micro-

scopic characters differ in no significant respect from those described and illustrated by Lagerheim in his original publication (Jahrb. Wiss. Bot. **24**: 549–565. 1892) except that no oidia were observed.

The only other species of *Dipodascus* is *D. uninucleatus,* recently described from Canada by Biggs (Mycologia **29**: 34–44. 1937). This lacks oidia but its growth characters and general habit seem to mark it as clearly distinct from the Colombian material. The nuclear condition, which is the fundamental basis for separating the two species, cannot, of course, be determined satisfactorily from dried material.

TRICHOCOMA PARADOXA Jungh.

This species is widely distributed in the warmer parts of both hemispheres but is apparently not common. Dodge (Ann. Myc. **27**: 152. 1929) notes collections in the new world from South Carolina, Puerto Rico and Brazil only. To this list may be added Panama. It was growing laterally on a fallen, mossy log in the forest south of the Llanos del Volcan, Province of Chiriqui, 1200–1350 m. (July 14, 1935. G.W.M. *2792*). Both Dodge and Boedijn (Ann. Jard. Buitenz. **44**: 243. 1935) agree that there is but a single species so far as known. The genus was made the type and only representative of the family Trichocomaceae by E. Fischer (Engler and Prantl 1^1: 310. 1897). Boedijn, on the basis of careful study, includes the genus in the Onygenaceae. Clements and Shear (Genera of Fungi 144. 1931) had previously done the same thing, but place the family in the Tuberales, whereas Boedijn retains it in the Plectascales. So far as can be decided by examination of mature specimens, Boedijn's disposition of the family is distinctly preferable.

Spore measurements, according to Dodge, are $6 \times 5\ \mu$; Boedijn gives them as $5.5–7 \times 3.5–6\ \mu$. In the Panama collection the fully matured spores measure $7–7.5 \times 5–6.5\ \mu$, averaging $7.4 \times 6.1\ \mu$. Numerous smaller immature spores were present in all mounts but could be eliminated by their capacity to take a phloxine stain, while the mature spores retained their bright brown color.

HYALORIA PILACRE A. Möller

This species, described by Möller (Protobasidiomyceten 137.

1895) from Brazil, was until recently the sole representative of its genus and family and the only known angiocarpous fungus with cruciate-septate basidia, corresponding in its angiocarpous character with the Phleogenaceae of the auriculariacean series. S. Killermann has recently described from Germany (Ber. Deutsch. Bot. Gesell. **54**: 165. 1936) what he regards as a second species of the genus, illustrating it with photographs which convey almost nothing and drawings which are, to say the least, crude and confusing. He did, however, examine what he believes to be one of Möller's specimens and notes certain helpful corrections to Möller's description.

What I regard as Möller's species was found on a decaying palm stem in the Sierra Nevada de Santa Marta (Hacienda Cincinnati, 1250–1500 m., Aug. 22, 1935. G.W.M. *3616*). The fructifications are much smaller than those described by Möller, few exceeding 1 cm. in height and the majority under 5 mm., and occur singly or in small groups rather than in the dense clusters he illustrates (l.c. pl. 1, f. 3) but microscopically the agreement is satisfactorily close.

Study of soaked specimens, supplemented by that of a few basidiocarps preserved in formal-acetic alcohol and imbedded, sectioned and stained with iron-alum haematoxylin permits certain additions and corrections to be made to our knowledge of this unique species.

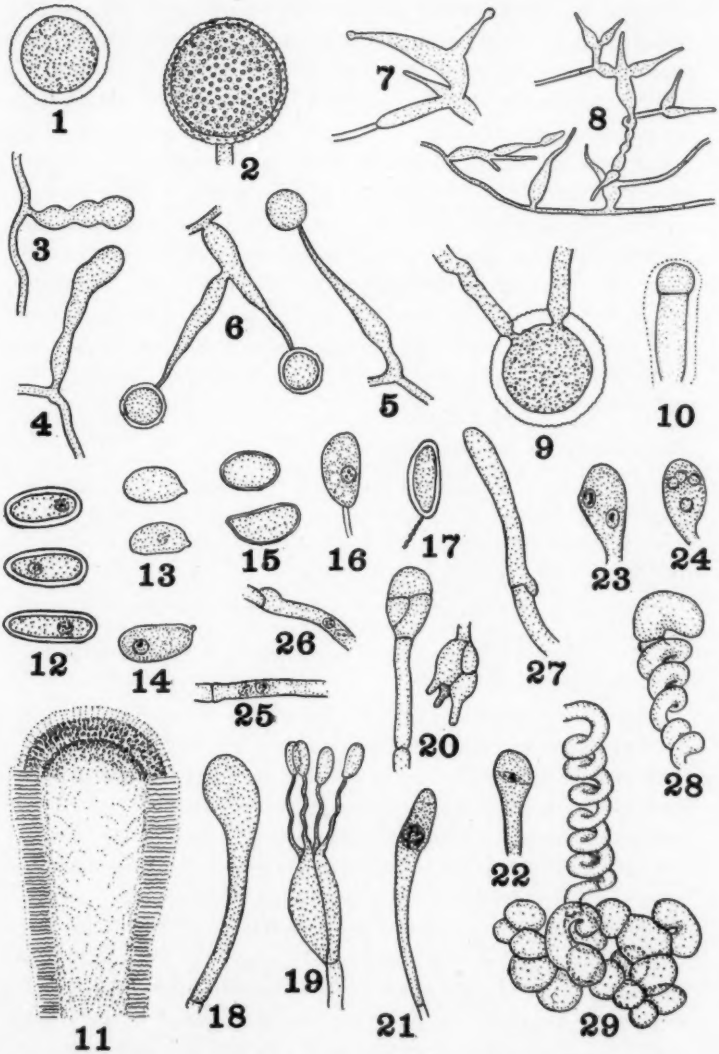
The dried basidiocarps are ochraceous yellow, extremely hard and horny, but the stalk and head are sharply defined. They soak up readily, becoming pure white, as they were when collected. The head remains relatively firm and opaque, the stalk somewhat less so, and the whole is surrounded by an envelope of transparent jelly which is thick about the stalk but much thinner above (FIG. 10). Longitudinal median sections of the preserved material (FIG. 11) show the body of the stalk to be composed of irregularly arranged hyphae with only a suggestion of longitudinal orientation and much interhyphal space. At the periphery, especially above, there is a fairly dense layer of coarse hairs. The hymenium occupies a dome-shaped area at the top and is composed of radiating, long-stalked, pyriform basidia, interspersed with long, sterile hairs which project above the basidia to a height somewhat less

than that of the hymenial layer. Beneath the hymenium is a very dense subhymenial layer, containing numerous swollen, vesicular cells, which gradually merges with the stalk below.

Möller (l. c. 138) refers to the hymenium, and again, in the formal description (p. 173) says: " Die tief unter der Oberfläsche, aber in einer Schicht angelegten Basidien. . . ." Gäumann (Vergl. Morph. Pilze 485. 1926) says, however, that the basidia are arranged irregularly, " nicht zu Hymenium vereinigt." Since Gäumann's account is based on that of Möller, it is difficult to discover the justification for such a statement. Certainly, in *Hyaloria* the hymenium is quite as clearly defined as it is in the majority of the tremellaceous fungi.

Möller describes the spores as " länglich ovalen," 7 μ in length, and notes the lack of apiculi. Killermann, in reexamining them, found them to be 8–10 $\times$ 4 μ. They occur in a dense mass above the hymenium, but retained within the gelatinous upper layer, and are wholly unlike the spores of other tremellaceous fungi. I find them 8.5–10 (–12) $\times$ 4–4.5 (–5) μ, the homogeneous interior surrounded by a hyaline, refractive wall up to 1 μ thick (FIG. 12). Stained spores show a single nucleus, sometimes in the center, but often at one end. Young basidiospores can be found, however, which are thin-walled and with a definite apiculus (FIG. 13, 14) as well as various intermediate stages between such young spores and the mature, thick-walled spores (FIG. 15), suggesting that after separation from the basidia the spores continue to develop at the expense of the gelatinous matrix in which they are imbedded, somewhat as in certain Gasteromycetes, although there is no evidence of nurse hyphae. The transformation of the spore may begin while it is still attached to the epibasidium and in spite of the definite apiculus, it is obvious that the spores are not violently discharged, but are detached by the breaking of the epibasidium some distance below the point of attachment (FIG. 17). This, again, is suggestive of what happens in many Gasteromycetes, and serves to emphasize the true angiocarpous nature of the species.

The basidia, as stated, are extraordinarily long-stalked at first (FIG. 18). Later a septum appears at the base of the enlarged terminal portion and four longitudinal segments are cut out, each



FIGS. 1–9, *Haplosporangium lignicola*; 10–27, *Hyaloria Pilacre*; 28–29, *Hobsonia gigaspora*.

of which produces a rather slender epibasidium (FIG. 19) which, unlike those of most of the Tremellales, bears no clearly defined sterigma at the tip (FIG. 16). While the great majority of the basidia are typically cruciate-septate, irregularities in septation are not uncommon (FIG. 20).

Cytologically, the development, so far as followed, is in entire accord with that of other members of the group. A large fusion nucleus (FIG. 21) undergoes two successive divisions, resulting in four nuclei (FIG. 22–24), one of which presumably enters each spore, although the actual passage has not been seen. The mycelium is regularly binucleate (FIG. 25, 26). Clamp connections were not certainly seen in the subhymenium or the interior of the stalk, but may readily be observed on the peripheral hairs of the stalk (FIG. 26, 27).

HELICOSPORIUM LUMBRICOPSIS Linder.

In moist chamber on decaying palm stem collected at edge of Llanos del Volcan, Chiriqui, about 1350 m., July 14, 1935. G.W.M. *2791* b. Reported by Linder (Ann. Missouri Bot. Gard. **16**: 284. 1929) from Florida, Venezuela and British Guiana. The " faint, pink tinge " mentioned by Linder was very apparent in the living fungus.

HOBSONIA GIGASPORA Berk.

Exceedingly abundant in the mountains of Chiriqui above 1500 m. and in the Sierra Nevada de Santa Marta at 1500–2200 m. The favorite substratum seems to be decayed stems of bamboo in both localities, but the fungus occurs on various kinds of wood, and was collected once on dead palm leaves. The sporodochia, in the better developments, are strikingly cupulate, and the conidiophores, while sometimes merely loosely coiled as shown in Linder's figure (Ann. Missouri Bot. Gard. **16**: pl. 28, f. 8. 1929) are often tightly wound in a close spiral (FIG. 28). The proliferation of the spores begins at a very early stage in their development, explaining the curiously irregular, knotted appearance they present (FIG. 29). The identification of this and the preceding species has been verified by Dr. Linder.

STATE UNIVERSITY OF IOWA,
IOWA CITY

EXPLANATION OF FIGURES

With the exception of fig. 10, all outlined with camera lucida and reproduced at approximately the magnifications given in each instance. Unless otherwise stated, drawn from soaked material in 3 per cent KOH and Phloxine.

Figs. 1–9, *Haplosporangium lignicola*. 1, mature conidium of average size, × 1200; 2, conidium of exceptionally large size, immature, with thin wall, still attached to conidiophore, × 1200; 3, 4, developing conidiophores, × 550; 5, unbranched conidiophore with immature conidium, × 550; 6, branched conidiophore with two nearly mature conidia, × 550; 7, much-branched and proliferating conidiophore from old culture, × 550; 8, group of same, × 250; 9, germinating conidium, × 1200.

Figs. 10–27, *Hyaloria Pilacre* A. Möll. 10, sketch of soaked specimen, × 7; 11, Diagrammatic longitudinal section, drawn from slide of material fixed in formal-acetic-alcohol and stained with iron-alum haematoxylin, × 25; 12, three mature, thick-walled spores; 13, two young, thin-walled, apiculate spores; 14, same, from fixed and stained material; 15, two spores with slightly thickened walls, apiculus still evident in lower; 16, thin-walled spore still attached to end of epibasidium, fixed and stained; 17, thick-walled spore with shrivelled epibasidium still attached, fixed and stained; 18, young basidium; 19, older basidium; 20, two basidia showing aberrant septation; 21, young basidium with fusion nucleus, fixed and stained; 22, first division of fusion nucleus, fixed and stained; 23, 24, two- and four-nucleate stages, fixed and stained; 25, internal mycelium with dicaryon, fixed and stained; 26, peripheral hair, with dicaryon and clamp connection, fixed and stained; 27, tip of peripheral hair with clamp connection. Figs. 12–27, × 1200.

Figs. 28–29, *Hobsonia gigaspora* Berk. G.W.M. *3758* (Colombia). 28, conidiophore, with conidial primordium; 29, later stage, showing proliferation of primordium. Figs. 28–29, × 550.

STUDIES IN THE SEXUALITY OF THE HETEROBASIDIAE [1]

HORACE L. BARNETT [2]

(WITH 3 FIGURES)

In the present work an attempt has been made to study the growth in culture and the sexuality of various species of the orders Auriculariales and Tremellales. The species included were *Auricularia Auricula-Judae* (Fries) Schröt., *Exidia glandulosa* (Bull.) Fries, *E. recisa* (Ditm.) Fries, *E. saccharina* Fries and *E. nucleata* (Schw.) Burt.

The literature concerning the Heterobasidiae, other than from a taxonomic standpoint, is scarce. Several writers have reported the germination of spores in culture for some of the species. Shear and Dodge (**16**) and Kniep (**10**) are the only investigators who have reported work on the sexuality of any species of this group. Cytological work has been published by Rogers (**14**, **15**), Neuhoff (**12**), and Whelden (**23**, **24**, **25**, **26**).

MATERIALS AND METHODS

The collections of the species studied in culture and from which monospore cultures were obtained are shown in Table 1.

In the records of spore sizes in Table 1, the first and last numbers are the extremes and the numbers in parentheses are the means. Twenty spores of III, VI and VII, and 100 spores each of I, IV, V and VIII were measured.

Two fruit bodies from collection V of *A. Auricula-Judae* were selected and designated as Va and Vb. These had already been

[1] This paper represents an abbreviation from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany of Michigan State College, East Lansing, Michigan.

[2] The writer wishes to acknowledge his indebtedness to Dr. E. A. Bessey under whose supervision these investigations were conducted.

removed from the host wood when received and it was not possible to determine how far apart they had been growing. From *E. glandulosa* II, two fruit bodies (IIa and IIb) were selected from the same log at a distance of two inches apart. The only collection of *E. saccharina* was growing on a stick about 12 inches long. Two fruit bodies from this were selected and designated as Ia and Ib. Only one fruit body was selected from each of the other collections.

TABLE 1

DATA CONCERNING THE COLLECTIONS USED THROUGHOUT THE EXPERIMENTS

Species	Collection number	Place of collection	Date	Host	Spore size
A. Auricula-Judae	I	Adirondacks, N. Y.[3]	8/23/35 Received	Fir?	10.5–(14.6)–17.5×4.8–(5.4)–7 μ
"	III	Lincoln, Nebr.[4]	11/7/35	Deciduous	11.8–(13.5)–15.5×4.8–(5.2)–5 7μ
"	IV	Chapel Hill, N. C.[5]	7/6/35 Received	Oak	8.8–(11.9)–14.8×5.2–(5.6)–6.6 μ
"	V	Lincoln, Nebr.[4]	10/28/35	Deciduous	8.8–(12.4)–14.4×4.4–(5.2)–6 μ
"	VI	Iowa City, Iowa[6]	9/2/36	"	10.5–(13.1)–15.2×4.8–(5.2)–5.7 μ
"	VII	Iowa City, Iowa[6]	9/3/36	Ash	11.3–(13.3)–14 ×4.8–(5.5)–6.2 μ
"	VIII	Fort Collins, Colo.[7]	1/5/37 Received	Fir	13.2–(15) –17.5×4.4–(5.4)–6.6 μ
E. glandulosa	I	E. Lansing, Mich.	11/13/34	Hickory	
"	II	E. Lansing, Mich.	4/29/36	"	
"	III	Lyons, Mich.	5/30/36	Oak	
E. recisa	I	E. Lansing, Mich.	3/28/36	Hickory	
"	II	Lyons, Mich.	5/30/33	Oak	
"	III	E. Lansing, Mich.	6/4/36	Hickory	
E. saccharina	I	Grayling, Mich.	9/10/36	White Pine	
E. nucleata	I	E. Lansing, Mich.	7/9/35	Maple	

[3] Collected by Dr. E. A. Bessey.
[4] By courtesy of Dr. Leva B. Walker.
[5] By courtesy of Dr. W. C. Coker.
[6] By courtesy of Dr. G. W. Martin.
[7] By courtesy of Prof. J. L. Forsberg.
All other specimens were collected by the author.

The most satisfactory method of obtaining spore deposits was similar to that used by Mounce (**11**). A portion of a fruit body was suspended from the cover of an agar plate and the spores were distributed by rotation of the cover. Well-isolated germinated spores were located by the high power objective of a binocular dissecting microscope. The spores and bits of the surrounding agar were picked out by the use of the eye of a specially prepared sewing needle and transferred to tubes of agar.

The best culture media for vegetative growth of all species were

potato dextrose agar and malt extract agar. The best media for the production of fruit bodies were potato dextrose agar with 1 gm. of peptone per liter, malt extract agar and prune-corn meal agar. The most typically shaped fruit bodies of *A. Auricula-Judae* were produced on moist, autoclaved fresh sticks of basswood and hickory.

RESULTS OF EXPERIMENTS

GERMINATION OF BASIDIOSPORES

The basidiospores of all species studied were one-celled at the time of discharge from the fruit body but some of the spores became two- or three-celled before germination (FIG. 1: 1, 10; FIG. 2: 1). The spores of all species, except *E. nucleata*, germinated readily and well on agar. In *A. Auricula-Judae* three methods of germination were observed. On agar they germinated by long stout germ tubes (FIG. 1: 3, 4) which grew directly into mycelium. Spores which fell back onto the fruit body produced secondary spores (FIG. 3: 4), similar in shape to the basidiospores. In distilled water the spores produced short slender germ tubes bearing sickle-shaped oidia (FIG. 1: 2*A, B*). In *E. glandulosa* the same three methods of germination were observed. In the same order these are illustrated by fig. 1: 12, 13, by fig. 1: 11 and by fig. 1: 9*A, B, C*. The same three methods were also observed in *E. nucleata* (FIG. 3: 1, 2, 3). Spores of *E. recisa* germinated only by means of long stout germ tubes (FIG. 2: 2, 3) which, in some cases, bore clusters of straight or slightly curved oidia (FIG. 2: 3). These oidia germinated on agar by means of secondary oidia or by producing normal mycelia directly (FIG. 3: 7). The method of germination in *E. saccharina* (FIG. 2: 10; FIG. 3: 5) was the same as in *E. recisa*. The oidia borne on the germ tubes germinated readily to produce normal monocaryon mycelia.

Fusions between young hyphae from two germinating spores were seen in *E. glandulosa* (FIG. 1: 17), *E. recisa* and *E. saccharina* (FIG. 2: 10). Abundant clamp connections were produced on dicaryon mycelia of all species included in this paper. These are illustrated as follows: *A. Auricula-Judae* (FIG. 1: 7), *E. glandulosa* (FIG. 1: 14), *E. recisa* (FIG. 2: 5), *E. saccharina* (FIG. 2: 6) and *E. nucleata* (FIG. 2: 13).

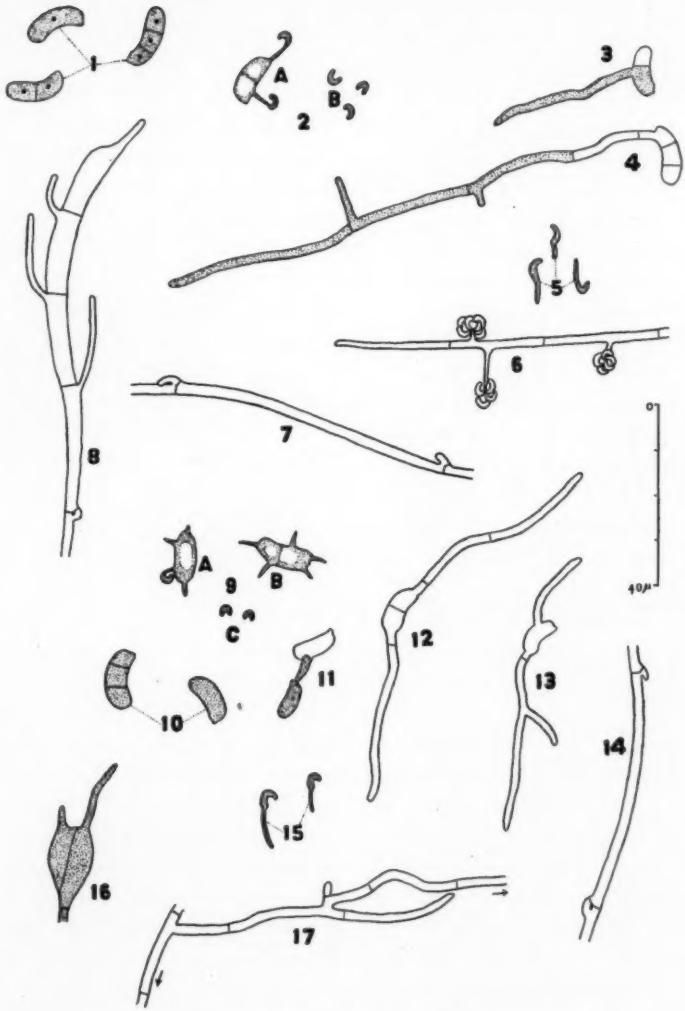


FIG. 1. *Auricularia Auricula-Judae* and *Exidia glandulosa*.

PRODUCTION OF OIDIA ON MYCELIA

Sickle-shaped oidia were borne in clusters on monocaryon mycelia of *A. Auricula-Judae* (FIG. 1: 6) and *E. nucleata* (FIG. 3: 6) and were also observed in small numbers in some of the monospore cultures of *E. glandulosa*. The oidia of these species were subjected to various conditions but no germination beyond the first stages were observed in *A. Auricula-Judae* (FIG. 1: 5), *E. nucleata* (FIG. 2: 12) and *E. glandulosa* (FIG. 1: 15). Oidia were observed only rarely in monocaryon cultures of *E. recisa*. Large masses of uninucleate oidia (FIG. 2: 8) were produced by most of the monospore cultures of *E. saccharina*. They were borne singly or in loose clusters (FIG. 2: 9), and germinated readily on agar to produce normal monocaryon mycelia. *E. saccharina* was the only species in which oidia were observed on dicaryon mycelia. In this case binucleate oidia (FIG. 2: 7) were borne singly on hyphae with clamp connections (FIG. 2: 6). Upon germination of these oidia clamp connections were formed on the first hyphae produced (FIG. 2: 11). From these, 37 single-oidial clusters were obtained. All produced normal dicaryon mycelia with clamp connections.

PRODUCTION OF FRUIT BODIES IN CULTURE

Dicaryon cultures of all species were grown on various media and under various conditions of temperature, light and moisture. Only one culture of *A. Auricula-Judae* and transfers from it developed fertile but atypical fruit bodies on agar (FIG. 3: 8). Normal basidia with four epibasidia (FIG. 1: 8) were found in young fruit bodies which later produced typical viable spores. Fruit bodies which were more typical in shape but which remained sterile (FIG. 3: 9) were developed on sterilized sticks of basswood and hickory.

In *E. recisa* a few typical four-celled basidia (FIG. 2: 4) were seen in only one dicaryon culture. In this case only a thin gelatinous layer was produced on the surface of the agar. No spores were seen. In several cultures (both dicaryon and monocaryon) there developed rounded, dark brown gelatinous bodies (FIG. 3: 10) which closely resembled normal young fruit bodies in appearance. It is thought that these gelatinous structures represented abortive fruit bodies which failed to develop basidia.

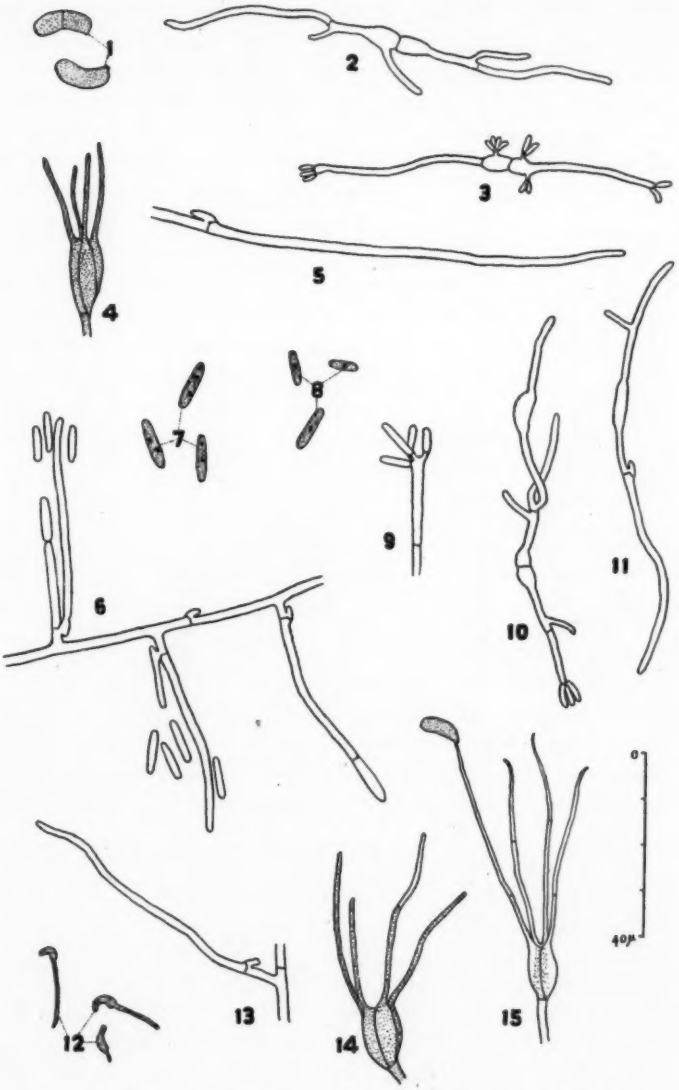


FIG. 2. *Exidia recisa, E. saccharina* and *E. nucleata.*

No signs of fruit body formation were seen in any dicaryon culture of *E. glandulosa*. Many young basidia were found in very small, gray gelatinous bodies in two monocaryon cultures. Several basidia were two-celled and rarely a four-celled basidium was seen. A few had produced two epibasidia (FIG. 1: 16) and a few typical basidiospores were seen in one culture. Although these cultures were checked thoroughly, using nuclear stains, no clamp connections were seen and all cells observed were uninucleate. This appears to be a case where fertile but quite atypical, poorly formed fruit bodies were produced by monocaryon mycelia.

Small white, cushion-like fruit bodies were produced in dicaryon cultures of *E. nucleata*. This was true for the multispore cultures and for some of the compatible pairings of monocaryon mycelia. Many four-celled typical basidia were present (FIG. 2: 14, 15). The longer epibasidia were often one or two-septate (FIG. 2: 14, 15). Typical viable spores were numerous on these fruit bodies. No signs of a central crystal body were seen in any of the fruit bodies in culture.

SEXUALITY

Because the species studied in the present work showed no other distinguishable difference between monocaryon and dicaryon mycelia and because the production of fruit bodies was rare, the presence of clamp connections alone was taken as an indication of true dicaryon mycelium. By staining mycelia both with and without clamp connections early in the course of this investigation, the correctness of this assumption was determined for the species in question.

The monospore cultures from each fruit body were given Arabic numbers, but without duplication of the numbers in other fruit bodies. Each fruit body was given a Roman numeral corresponding to the number of the collection. The monospore cultures from each fruit body were paired in all combinations on agar slants in 15 mm. test tubes. After about two weeks the line of contact between the two mycelia was examined for the presence of clamp connections. When clamps were found the two mycelia were said to be compatible. In several cases short branches were seen at septa and these may have been abortive clamp connections, but un-

less complete and definite clamps were seen the results were considered as negative. When irregular results were obtained the pairings were repeated. In all tables the + sign indicates the presence of clamp connections and the — sign indicates their absence.

A. AURICULA-JUDAE

Twenty-two monospore cultures from fruit body I were paired in all possible combinations and the results are given in Table 2. The cultures fell into two groups, based upon the production of

TABLE 2

A. Auricula-Judae. RESULTS OF PAIRING 22 MONOSPORE CULTURES OF FRUIT BODY I

Pairings between members of the same sexual phase are not shown.

	4	5	6	8	9	10	12	13	15	16	17	19	22	24
1	+	+	+	+	+	−	+	−	+	+	+	+	−	+
3	+	−	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	−	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	−	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+

clamp connections in certain combinations. Throughout this paper these groups are referred to as " sexual phases." The two sexual phases were designated as A and a. When members of the same sexual phase were paired no clamp connections were produced. Clamp connections were found in all but six of the 112 pairings between A and a.

The monospore cultures of each of the other fruit bodies were paired in all combinations as follows: 19 from III, ten from III F_1 (a fruit body grown in culture), 11 from IV, 11 from Va, seven from Vb, nine from VI, six from VII and nine from VIII. The

results of the pairings of fruit body IV are given in Table 3. In all these fruit bodies, except VIII, the results were very regular. Clamp connections were found in every pairing between members of different sexual phases while none were present in any pairing between members of the same sexual phase. The results of pairings of cultures from fruit body VIII showed that clamp connections were present in only one (502 × 505) of the 36 pairings. Clamps were present in the fruit body but none were found in a culture arising from many spores. The results obtained from fruit

TABLE 3

A. Auricula-Judae. RESULTS OF PAIRING 11 MONOSPORE CULTURES OF FRUIT BODY IV

· = presence of aversion.

	151	152	155	157	158	161	153	154	156	159	160
151		−.	−.	−.	−	−	+	+	+	+	+
152			−.	−.	−.	−.	+	+.	+	+	+
155				−.	−.	−.	+	+	+	+	+
157					−.	−.	+	+.	+	+	+
158						−.	+	+	+.	+	+
161							+	+	+	+	+
153								−.	−.	−.	−.
154									−.	−.	−.
156										−.	−.
159											−.
160											

body VIII are somewhat puzzling. The fact that one pairing produced clamp connections would indicate that both sexual phases were represented in the nine monospore cultures used. However, if this is true, there must exist some condition which causes a very low degree of compatibility between the two sexual phases. These results are similar to those reported by Kniep (**10**) for *Auricularia mesenterica.* He found a fruit body which showed clamp connections but mycelium grown from a mass of spores produced no clamps.

From the foregoing it will be noted that all fruit bodies, except number VIII, showed distinctly the presence of two sexual phases, and that all of these, except for six pairings in I, showed normal regular compatibility between the two sexual phases. Such results indicate that *A. Auricula-Judae* is "heterothallic" and typically bipolar.

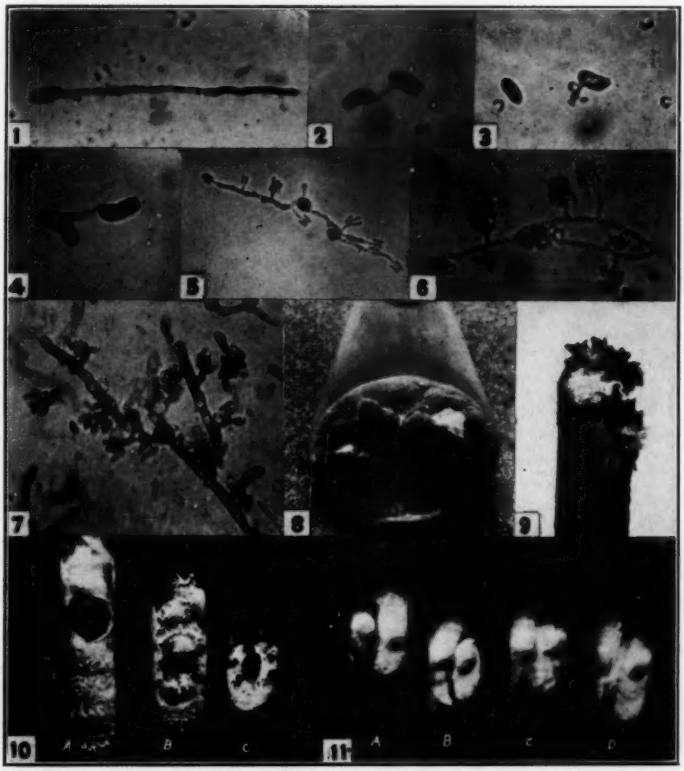


FIG. 3. *Auricularia* and *Exidia*.

In most cases when pairings were made between different fruit bodies, two monospore cultures from each sexual phase were chosen from each fruit body and mated with those of the other fruit bodies. In some cases where results were irregular more

cultures were used. In all tables the brackets connect the cultures of the same sexual phase. The results of these pairings fell into four different types. (1) Every pairing was compatible. This was the case in all combinations of fruit bodies III, IV, Va, VI and VII, and also of Va × Vb and VII × VIII. The cross of III × IV is given as an example in Table 4. (2) When pairings were made between fruit body I and fruit bodies III, IV, VI and VII, all were incompatible. The results of the cross I × III are given as an example in Table 5. (3) In some of the crosses involving I and VIII some of the pairings were compatible while

TABLE 4

A. Auricula-Judae. RESULTS OF PAIRINGS BETWEEN FRUIT BODIES III AND IV

		III			
		A_1		a_1	
		101	105	104	112
IV	A_2 151	+	+	+	+
	A_2 152	+	+	+	+
	a_2 153	+	+	+	+
	a_2 154	+	+	+	+

TABLE 5

A. Auricula-Judae. RESULTS OF PAIRINGS BETWEEN FRUIT BODIES I AND III

		III			
		A_1		a_1	
		101	105	104	112
I	A 3	−	−	−	−
	A 7	−	−	−	−
	a 4	−	−	−	−
	a 6	−	−	−	−

others were incompatible. This was true of I × Va (Table 6), I × VIII (Table 7), III × VIII (Table 8) and IV × VIII. In the last cross only one out of 20 pairings was compatible. In all these the compatible pairings were scattered and did not correspond to any certain combinations of sexual phases. (4) In the cross III × III F_1, clamp connections were found in just half of the pairings and only in certain combinations of sexual phases, indicating that the two sexual phases of III F_1 were identical with those of III. In the first, second and third types the results show that no two sexual phases involved are identical. This implies the conception that within this species, several or many sexual phases exist, only two such phases being present in the same fruit body, and that only one pair of chromosomes is involved. This view

TABLE 6

A. Auricula-Judae. RESULTS OF PAIRINGS BETWEEN FRUIT BODIES I AND Va

Va

I		A_3				a_3			
		201	205	207	208	203	204	206	211
A	1	−	−	−	−	−	−	−	−
	3	−	−	+	+	−	−	−	−
	7	−	−	−	−	−	−	+	−
	18	−	−	−	−	−	+	−	+
a	4	+	−	−	−	+	−	−	−
	6	−	−	−	+	+	+	−	−
	8	−	−	−	−	+	−	−	−
	9	−	−	−	−	−	−	−	−

TABLE 7

A. Auricula-Judae. RESULTS OF PAIRINGS BETWEEN FRUIT BODIES I AND VIII

VIII

I		501	502	503	504	505
A	3	−	+	+	−	−
	7	+	+	+	+	+
a	4	+	+	+	+	+
	6	+	+	+	+	+

TABLE 8

A. Auricula-Judae. RESULTS OF PAIRINGS BETWEEN FRUIT BODIES III AND VIII

VIII

III		501	502	503	504	505
A_1	101	+	−	+	+	−
	105	+	−	+	+	−
a_1	104	+	−	+	+	−
	112	+	−	+	+	−

supposes the presence of the compatibility factors as multiple allelomorphs.

A summary of all the pairings of monospore cultures is given in Table 9. The + sign indicates that all pairings were compatible, while the — sign indicates that all pairings were incompatible. The ± sign indicates that some of the parings were compatible and others were incompatible.

In the tables the genetical designation of the sexual phases (A, a, A_1, a_1, etc.) is entirely arbitrary. The letters with subscripts

represent allelomorphs of A and a. Following Kniep, it is assumed that the presence of two identical compatibility factors results in incompatibility and that compatibility results (*i.e.* clamp connections are produced) when two somewhat different factors of the allelomorphic series are present. It is further assumed that incompatibility also results if there is too great a difference be-

TABLE 9

A. Auricula-Judae. SUMMARY OF RESULTS OF ALL PAIRINGS OF MONOSPORE CULTURES

		I		III		III F1		IV		Va		Vb		VI		VII		VIII
		A	a	A_1	a_1	A_1	a_1	A_2	a_2	A_3	a_3	A_4	a_4	A_5	a_5	A_6	a_6	
I	A	−	+	−	−			−	−	±	±			−	−	−	−	±
	a		−	−	−			−	−	±	±			−	−	−	−	+
III	A_1			−	+	−	+	+	+	+	+			+	+	+	+	±
	a_1				−	+	−	+	+	+	+			+	+	+	+	±
III F1	A_1					−	+											
	a_1						−											
IV	A_2							−	+	+	+			+	+	+	+	−
	a_2								−	+	+			+	+	+	+	±
Va	A_3									−	+	+	+	+	+	+	+	
	a_3										−	+	+	+	+	+	+	
Vb	A_4											−	+					
	a_4												−					
VI	A_5													−	+	+	+	
	a_5														−	+	+	
VII	A_6															−	+	+
	a_6																−	+
VIII																		±

tween the two factors. This latter assumption may serve as a partial explanation for the low degree of compatibility between two fruit bodies, but it also seems quite possible that some other factors or conditions are present which influence the compatibility between fruit bodies.

No special work was done with the phenomenon of aversion but it was observed in a number of pairings, especially of fruit body IV (Table 3). Of the 55 pairings three types were observed:

(1) 22 (one compatible pairing) showed space of aversion (FIG. 3: 11*A* and *B*); (2) two (both compatible) showed aversion in which the gap was partially filled with dicaryon mycelium (FIG. 3: 11*D*); (3) 31 (29 compatible pairings) showed even intermingling of the mycelia (FIG. 3: 11*C*). These results indicate that in this fruit body there was a rather close correlation between the presence of aversion and incompatibility of the pairings. Aversion in pairings of mycelia from other fruit bodies was observed only in a few cases and its presence showed no correlation with incompatibility.

The same method used in pairing monospore cultures of *A. Auricula-Judae* was followed for the other species.

E. GLANDULOSA

Monospore cultures from the same fruit body were paired as follows: 15 from I, nine from IIa, ten from IIb and ten from III. The results in every case indicated normal bipolarity. No irregular results were found. In the pairings between fruit bodies, four monospore cultures from each were used. The cross IIa × IIb was repeated using eight cultures from IIa and all ten from IIb. A summary of all pairings is given in Table 10. The results

TABLE 10

E. glandulosa. SUMMARY OF RESULTS OF ALL PAIRINGS OF MONOSPORE CULTURES

		I		IIa		IIb		III	
		A	a	A_1	a_1	A_2	a_2	A_3	a_3
I	A	−	+	+	+	+	+	+	+
	a		−	+	+	+	+	+	+
IIa	A_1			−	+	+	+	+	+
	a_1				−	+	+	+	+
IIb	A_2					−	+	+	+
	a_2						−	+	+
III	A_3							−	+
	a_3								−

show that no two of the sexual phases were identical. Thus, as in *A. Auricula-Judae,* the sexual phases are considered as being due to factors that exist as multiple allelomorphs. The genetical designations for each fruit body are given in Table 10.

When the pairings between fruit bodies IIa and IIb were examined, it was noticed that abundant clamp connections were present in some, while in others they were present in much smaller numbers. Preliminary experiments showed that the low numbers were limited to pairings between certain definite sexual phases, namely $A_1 \times A_2$ and $a_1 \times a_2$. To determine the quantitative differences in the number of clamp connections, pairings were made between all combinations of sexual phases, using three monospore cultures from each. Bits of two monocaryon mycelia were placed on malt agar plates at a distance of about 1 cm. apart and incubated at room temperature. In order to make the conditions uniform it was necessary to know the exact time at which the two mycelia came into contact. This was accomplished by placing sterilized halves of cover glasses into the agar in an upright position between the two mycelia. The mycelia were allowed to grow up against the cover glass which was then removed. At the end of 48 hours after the removal of the intervening cover glasses the pairings were examined at the line of contact between the mycelia using the oil immersion lens. A drop of aqueous safranin was usually added to make the hyphae more distinct. Areas were chosen in which the hyphae could be seen clearly and in which their number was about average, and counts were made of the clamp connections in the entire field, both on the surface and below the surface of the agar as far as the focus of the lens would reach. Ten counts were made from each pairing between two monocaryon mycelia. The averages of these counts are given in Table 11. Table 12 gives the averages for each combination of sexual phases.

Certain distinct differences in reactions are evident. $A_1 \times a_1$, $A_2 \times a_2$ and $A_2 \times a_1$ produced approximately the same number of clamp connections, averaging 13.6, 13.0 and 13.0 respectively. $A_1 \times A_2$ and $a_1 \times a_2$ showed a decidedly smaller number, averaging 6.4 and 5.5 respectively. The average of $A_1 \times a_2$ was intermediate, being 10.3. When analyzed statistically, significant differences were found between the following pairs of combinations:

$A_1 \times A_2$ and $A_1 \times a_2$, $A_1 \times A_2$ and $A_1 \times a_1$, $A_1 \times A_2$ and $a_1 \times A_2$, $A_1 \times A_2$ and $A_2 \times a_2$, $a_1 \times A_2$ and $a_1 \times a_2$, $A_1 \times a_2$ and $a_1 \times a_2$, and $A_1 \times a_2$ and $A_1 \times a_1$.

Attempts were made to determine the exact cause of the differences in the number of clamp connections. It was found that in actively growing mycelia the number of septa without clamp connections was small and no distinct difference was observed in any of the mycelia. It was also thought that a difference might exist in the rate of diploidization of one monocaryon mycelium by another. The method employed was similar to the one used by Buller (**5**). Bits of mycelium from cultures 28 and 30 were used to diploidize sets of cultures 53, 55, 52 and 57. Conversely, bits of mycelium cultures 53, 55, 52 and 57 were used to diploidize cultures of 28 and 30. The cultures in each case were 13 days old. At the end of seven days after diploidization, examination showed the presence of clamp connections in every case at a distance of 1 cm. from the point of diploidization. This distance, however, was short enough to be accounted for by the radial growth of the

TABLE 11

E. glandulosa. RESULTS OF CLAMP CONNECTION COUNTS IN PAIRINGS BETWEEN FRUIT BODIES IIa AND IIb

The numbers represent the average number of clamps per microscopic field.

		A_1			a_1			a_2		
		21	23	24	27	28	30	51	52	57
A_2	50	5.5	5.1	7.5	11.8	13.1	13.1	13.9	14.3	12.4
	53	7.3	6.1	7.1	14.6	16.4	11.4	14.7	13.5	14.6
	55	4.8	7.9	6.1	11.3	12.6	13.1	12.7	12.0	8.8
a_2	51	11.6	12.8	9.2	5.2	7.5	5.0			
	52	10.0	10.5	8.8	6.5	5.0	5.0			
	57	9.8	10.5	9.6	5.3	4.9	5.3			
a_1	27	15.3	17.1	10.9						
	28	11.9	13.2	12.2						
	30	12.3	16.9	12.5						

TABLE 12

E. glandulosa. SUMMARY OF RESULTS GIVEN IN TABLE 11

	A_1	a_1	a_2
A_2	6.4	13.0	13.0
a_2	10.3	5.5	
a_1	13.6		

dicaryon mycelia, which ranged from 12 to 16 mm. in the seven-day period. In three cases clamp connections were found at a distance of 2 cm. and in two cases at a distance of 3 cm. These results show that the rate of diploidization cannot serve as an explanation for the differences found in the number of clamp connections.

It seems possible that there may be a greater attraction between certain mycelia (if such an attraction exists at all) and as a result the union of mycelia may take place sooner than if a lesser attraction exists. Consequently, a greater number of clamp connections would be formed within the 48 hours that elapsed between the removal of the cover glass from between the mycelia and the counting of the clamp connections. This, however, is purely suppositional and is offered merely as a possible explanation for the differences found. As yet, no successful attempts have been made to demonstrate the presence of such an attraction between compatible mycelia.

E. RECISA

Monospore cultures from the same fruit body were paired as follows: 20 cultures from fruit body I, 10 from II and 10 from III. In all cases the results were very regular and showed the presence of two sexual phases. Four monospore cultures from each fruit body were used in the pairings between different fruit bodies. All pairings between members of any two sexual phases were compatible. A summary of the results of all pairings is given in Table 13. Thus, as in *A. Auricula-Judae* and in *E. glandulosa,* there are present in *E. recisa* a number of sexual phases which are thought to exist as multiple allelomorphs.

E. SACCHARINA

Two fruit bodies were selected from the only collection of this species and designated as Ia and Ib. Seven monospore cultures of Ia were paired in all combinations as were 16 cultures of Ib. The results were very regular, showing the presence of two sexual phases in each fruit body. Four monospore cultures of Ia were paired with four cultures of Ib and the results showed the pres-

TABLE 13

E. recisa. SUMMARY OF RESULTS OF ALL PAIRINGS OF MONOSPORE CULTURES

		I		II		III	
		A	a	A_1	a_1	A_2	a_2
I	A	−	+	+	+	+	+
	a		−	+	+	+	+
II	A_1			−	+	+	+
	a_1				−	+	+
III	A_2					−	+
	a_2						−

ence of clamp connections in every pairing. Again, as in *E. glandulosa,* complete compatibility was found to exist between two fruit bodies growing very near one another.

E. NUCLEATA

A few multispore cultures were obtained from one wild fruit body but owing to the very poor germination of the spores no monospore cultures were secured at this time. However, monospore cultures were later secured from fruit bodies produced in culture. These cultures grew very slowly and several of them either became contaminated or died out due to loss of vigor. Eleven cultures were paired in all combinations (with the exception of a few pairings) and the results are given in Table 14. The blanks in the table indicate no pairing because of the loss of cultures. Cultures 3, 14, 15, 17, 26 and 27 fell into one sexual phase and 12, 13 and 22 into a second sexual phase. All members of the first phase were compatible with all members of the second. Cultures 25 and 30, however, were exceptions to the normal polarity in their pairing reactions. These two cultures were incompatible with all the other monospore cultures and also with each other. It is not possible to tell from these results whether these groups represent three sexual phases of a quadripolar species, or whether *E. nucleata* is typically bipolar and that cultures

TABLE 14

E. nucleata. RESULTS OF PAIRING 11 MONOSPORE CULTURES
⊕ = fertile fruit bodies.

	3	14	15	17	26	27	12	13	22	25	30
3		−	−	−		−	+	⊕	+	−	−
14			−	−	−	−		⊕		−	−
15					−	−	+	⊕	⊕	−	−
17					−	−	+	+	⊕	−	−
26						−	+	⊕	+	−	−
27							+		⊕	−	
12									−	−	−
13									−	−	−
22										−	−
25											−
30											

25 and 30 were merely irregular in their compatibility. The later view seems more probable. The ⊕ sign indicates the production of fruit bodies with mature basidia and basidiospores.

Attempts were made to hybridize *E. recisa* with *E. glandulosa* and *E. saccharina* but all were unsuccessful.

DISCUSSION

The terms used by different authors in describing the sexuality of the Basidiomycetes vary to a certain extent. The term "sexes" has been applied by a number of investigators to the different groups into which the monospore cultures fall, based upon the production of clamp connections when the cultures from one fruit body are paired in all combinations.

Considering the fungi in general, the term "sexes" is most commonly used to distinguish two individuals, one of which acts as male and the other as female. This conception includes the passage of nuclei from only one individual (male) to the other (female). In many fungi, however, this is not the case. Buller (**6**) has shown that, in *Coprinus lagopus,* two compatible mono-

caryon mycelia may unite and there would follow an exchange of nuclei so that each mycelium becomes diploidized by the other. It seems reasonable to consider that in such fungi sexuality exists but with no distinction between sexes. For this reason the term "sexual phases," used by Bessey (**2**), is employed in this paper instead of "sexes" which is used by some authors.

Following Smith and Brodie (**17**) and Bessey (**2**), the terms "compatible" and "incompatible" are used throughout the present paper in the same sense that "fertile" and "sterile" are used by some other investigators. The term "compatible" is used to describe two monocaryon mycelia which unite, with subsequent production of dicaryon mycelium (indicated by the presence of clamp connections) and "incompatible" is used to describe two monocaryon mycelia which do not produce dicaryon mycelium.

The results obtained from pairing monospore cultures of the same fruit body of each species indicate that *A. Auricula-judae, E. glandulosa, E. recisa* and *E. saccharina* are typically bipolar. The pairing of monospore cultures of one fruit body of *E. nucleata* did not show typical bipolarity. Irregularities were found in pairings of monospore cultures of fruit bodies of I and VIII of *A. Auricula-Judae*. In I these were represented by constant incompatibility between certain monospore cultures of different sexual phases. Such irregularities or deviations from the normal polarity have also been reported by Kniep (**10**), Vandendries (**21, 22**), Brunswik (**4**) and Dickson (**7**) for various Hymenomycetes. As suggested by Dickson, there seem to be present some conditions or some additional factors which influence the compatibility of monospore cultures. Vandendries (**22**) considers that the tendency toward incompatibility is due to "une déficience des realisateurs sexueles." Kniep (**10**) attributes such deviations to quantitative gene changes.

It has been observed in the present work that within the same species complete compatibility was found between three collections of *E. glandulosa,* three collections of *E. recisa* and between five of the seven collections of *A. Auricula-Judae* (III, IV, V, VI and VII). In addition to the pairings between fruit bodies of different collections, complete compatibility was found to exist between two fruit bodies of *A. Auricula-Judae* (Va and Vb), taken

from the same collection. This was also true for two fruit bodies of *E. saccharina* (Ia and Ib) growing on the same stick within 12 inches of each other, and for two fruit bodies of *E. glandulosa* (IIa and IIb) growing but two inches apart. Similar complete compatibility between the monocaryon mycelia of two or more fruit bodies have been reported by Kniep (**10**), Brunswik (**4**), Newton (**13**), Vandendries (**18, 19, 20, 22**), Hanna (**8**), Mounce (**11**) and Arnold (**1**) for various Hymenomycetes.

Complete incompatibility was found in *A. Auricula-Judae* when cultures of fruit body I were paired with cultures from fruit bodies III, IV, VI and VII. Partial compatibility existed between fruit bodies I and Va, I and VIII, III and VIII, and IV and VIII. Fruit bodies I and VIII were collected from coniferous hosts and had longer spores on the average than those of other fruit bodies, which were collected from deciduous hosts. It is suggested that the variation in spore length, or the difference in the hosts, or both, may be associated with genetic differences great enough to influence the compatibility of the monocaryon mycelia. Similar cases of complete or partial incompatibility between fruit bodies of the same species have been reported by Kniep (**10**), Brunswik (**4**), Vandendries (**20**) and Mounce (**11**). Kniep (**10**) states that such incompatibility may be due to too great a quantitative difference in the genes or to secondary factors.

Several different theories of sexuality in fungi have been advanced to explain the complete compatibility found to exist between different fruit bodies of the same species. Among them are the theory of sexual mutations, Hartman's (**9**) theory of relative sexuality and Kniep's theory of multiple allelomorphs. The theory of Kniep seems to be the best for interpreting the results of the present work.

Considering that the compatibility factors exist as multiple allelomorphs, the question arises whether certain pairs of factors will produce a stronger degree of compatibility than others. A probable answer to this question was found when all monospore cultures of *E. glandulosa* IIa were discovered to be compatible with all cultures of IIb and a greater number of clamp connections were found in certain combinations of sexual phases than in others. The assumption is made in these experiments that a greater num-

ber of clamp connections indicates a stronger degree of compatibility between the monospore cultures involved. The results shown in Table 12 would then indicate a much stronger degree of compatibility between A_1 and a_1, A_2 and a_2, A_1 and a_2, and A_2 and a_1, than exists between A_1 and A_2, and a_1 and a_2. If the numbers of clamp connections can be considered, in this case, as representing the approximate quantitative differences between pairs of allelomorphs, it is found that the factors a_1 and a_2 are the most similar, with A_1 and A_2 only slightly less similar. The greatest differences are between A_1 and a_1, A_2 and a_2, and A_2 and a_1.

Diploidization rates were found to be about the same in pairings of $a_1 \times A_2$ and $a_1 \times a_2$. The variations that did occur were not sufficient to explain the differences found in the number of clamp connections. It is suggested that there may be a greater attraction between certain monocaryon mycelia than others, but as yet, no such attraction has been demonstrated.

POSTSCRIPT

Since the manuscript was sent to press a collection of *A. Auricula-Judae* (number IX) was received from Dr. F. A. Wolf of Durham, N. C. Monospore cultures from one fruit body showed typical bipolarity. When these cultures were paired with those from fruit bodies of other collections it was found that fruit body IX was completely compatible with fruit bodies III, IV and Va, partially compatible with VIII and completely incompatible with I.

Four additional monospore cultures from fruit body VIII were paired with the nine already obtained. Three of these pairings produced clamp connections.

DEPARTMENT OF BOTANY,
MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN

LITERATURE CITED

1. **Arnold, Jean D.** A comparative study of certain species of *Marasmius* and *Collybia* in culture. Mycologia **27**: 388–417. *pl. 35, 36.* 1935.
2. **Bessey, Ernst A.** A text-book of mycology. 495 pp. *139 fig.* P. Blakiston's Son and Co., Philadelphia. 1935.
3. **Bose, S. R.** Sexuality of *Polyporus ostrieformis* and *Polystictus hirsutus*. La Cellule **42**: 249–266. *1 pl.* 1933.

4. **Brunswik, H.** Untersuchungen über die Geschlechts- und Kernverhältnisse der Hymenomycetengattung *Coprinus*. Botanische Abhandlungen herausgegeben von Dr. K. Goebel **1**: 1–152. *fig. 1–3.* 1924.
5. **Buller, A. H. Reginald.** The biological significance of conjugate nuclei in *Coprinus lagopus* and other Hymenomycetes. Nature **126**: 686–689. *fig. 1–7.* 1930.
6. ——. Researches on fungi. Longmans, Green and Company. London and New York. Vol. IV. 329 pp. *4 pl., 149 fig.* 1931.
7. **Dickson, H.** Studies on *Coprinus sphaerosporus*. I. The pairing behavior and the characteristics of various haploid and diploid strains. Ann. Bot. **48**: 527–547. *pl. 11.* 1934.
8. **Hanna, W. F.** The problem of sex in *Coprinus lagopus*. Ann. Bot. **39**: 431–457. 1925.
9. **Hartmann, M.** Relative Sexualität und ihre Bedeutung für eine allgemeine Sexualitäts- und eine allgemeine Befruchtstheorie. Naturwiss. **19**: 8–19, 31–37. 1931. Cited by Bose (**3**).
10. **Kniep, Hans.** Die Sexualität der niederen Pflanzen. 544 pp. *221 fig.* Gustav Fischer. Jena. 1928.
11. **Mounce, Irene.** The biology of *Fomes pinicola* (Sw.) Cooke. Canada Dept. Agr. Bull. *111.* New Series. 75 pp. *pl. 1–10.* 1929.
12. **Neuhoff, W.** Zytologie und systematische Stellung der Auriculariaceen und Tremellaceen. Bot. Archiv **8**: 250–297. *pl. 1–4, fig. 1–7.* 1924.
13. **Newton, Dorothy E.** Bisexuality of individual strains of *Coprinus rostrupianus*. Ann. Bot. **40**: 105–128. *pl. 6, fig. 1–17.* 1926.
14. **Rogers, Donald P.** A cytological study of *Tulesnella*. Bot. Gaz. **94**: 86–105. *fig. 1–79.* 1932.
15. ——. Basidial proliferation through clamp formation in a new *Sebacina*. Mycologia **28**: 347–362. *fig. 1–34.* 1936.
16. **Shear, C. L. & B. O. Dodge.** The life history of *Pilacre faginea* (Fr.) B. & R. Jour. Agr. Res. **30**: 407–417. *pl. 1–2.* 1925.
17. **Smith, A. H. & H. J. Brodie.** Cultural characters and pairing reactions of monosporous mycelia and development of the fruit body of *Pholiota* (*Flammula*) *polychroa*. Bot. Gaz. **96**: 533–546. *30 fig.* 1935.
18. **Vandendries, René.** Nouvelle recherches sur la sexualité des Basidiomycètes. Bull. Soc. Bot. Belgique **62**: 133–136. 1924.
19. ——. Contribution nouvelle a l'étude de la sexualité des Basidiomycètes. La Cellule **35**: 129–157. *pl. 1–2, fig. 1–4.* 1925.
20. ——. Le comportment sexuel du *Coprinus micaceus* dans ses rapports la dispersion de l'espéce. Bull. Soc. Bot. Belg. **60**: 62–65. 1927.
21. ——. La bipolarité sexuelle chez *Coprinus disseminatus* Pers. Bull. Soc. Bot. Belg. **62**: 133–136. 1930.
22. ——. Nouvelle investigations dans le domaine sexuel des Hyménomycetes. Bull. Soc. Fr. **49**: 130–165. *7 fig.* 1933.
23. **Whelden, Roy M.** Cytological studies in the *Tremellaceae*. I. *Tremella*. Mycologia **26**: 415–435. *pl. 47–49.* 1934.

24. ——. ——. II. *Exidia.* Mycologia **27**: 41–57. *pl. 8–11.* 1935.
25. ——. ——. III. *Sebacina.* Mycologia **27**: 503–520. *fig. 1–3.* 1935.
26. ——. Observations on the cytology of *Sebacina globospora* n. sp. Rhodora **37**: 121–127. *1 text fig., pl. 331.* 1935.

EXPLANATION OF FIGURES

Fig. 1: 1–8, *A. Auricula-Judae.* 1, basidiospores; 2, *A,* basidiospore germinating in water, *B,* oidia produced by spores in water; 3, 4, basidiospores germinating on agar; 5, germinating oidia; 6, monocaryon hypha bearing oidia; 7, dicaryon hypha showing clamp connections; 8, basidium from fruit body in culture. 9–17, *E. glandulosa.* 9, *A, B,* basidiospores germinating in water, *C,* oidia produced by them; 10, basidiospores; 11, basidiospore producing secondary spore; 12, 13, basidiospores germinating on agar; 14, dicaryon hypha showing clamp connections; 15, germinating oidia; 16, two-celled basidium produced by monocaryon mycelium; 17, union of two young hyphae, arrows point toward germinating spores.

Fig. 2: 1–5, *E. recisa.* 1, basidiospores; 2, basidiospore germinating on agar; 3, same as 2 but producing oidia; 4, four-celled basidium produced on dicaryon mycelium; 5, dicaryon hypha. 6–11, *E. saccharina.* 6, dicaryon hyphae with oidia; 7, binucleate oidia from dicaryon mycelium; 8, uninucleate oidia from monocaryon mycelium; 9, monocaryon hypha bearing oidia; 10, union of young hyphae from two basidiospores; 11, germinating binucleate oidium. 12–15, *E. nucleata.* 12, germinating oidia; 13, dicaryon hypha; 14, 15, four-celled basidia with septate epibasidia from fruit body in culture.

Fig. 3: 1–3, germination of basidiospores of *E. nucleata,* × 750; 1, by a long germ tube; 2, by secondary spore; 3, by oidia. 4, *A. Auricula-Judae,* production of secondary spore, × 750. 5, *E. saccharina,* germinating basidiospore and oidia borne on germ tube, × 400. 6, *E. nucleata,* monocaryon hyphae bearing oidia, × 750. 7, *E. recisa,* germinating oidia and mycelium produced by them, × 750. 8, 9, fruit bodies of *A. Auricula-Judae,* × ⅕; 8, on agar, 9, on basswood stick. 10, *E. recisa,* gelatinous bodies in culture, *A,* dicaryon, *B, C,* monocaryon, × ⅕. 11, *A. Auricula-Judae,* pairings of monospore cultures of IV, *A,* aversion 155 × 157, *B,* aversion 157 × 161, *C,* even union of mycelia 157 × 160, *D,* aversion with gap filled with dicaryon mycelium 154 × 157, × ⅞.

NOTES AND BRIEF ARTICLES

A mimeographed program of the first Atlantic City meeting was mailed to the members of the Society in December 1932. Programs for subsequent meetings were printed. The Historian of the Society wishes to obtain a copy of this mimeographed program for preservation and binding as a part of the archives of the Society. He requests that any member who has preserved the program forward it to him for this purpose. He would also emphasize the desirability of members submitting photographs taken on the occasion of summer forays. It is hoped that in a few years the accumulation of such materials will provide an interesting exhibit which can be shown at one of the winter meetings.—H. M. FITZPATRICK.

A bumper crop of fleshy fungi appeared in central Florida the past summer, which kept the writer very busy collecting, describing, and testing various novelties; and teaching amateur mycophagists. *Lepiota Morgani,* for example, proved to be decidedly poisonous, though scarcely fatal. A thousand sporophores of this handsome plant appeared at once in a pasture and six of them were eaten at a meal. Among the boletes only one mildly poisonous species was found, *Boletus luridus,* but at least four were so decidedly bitter when cooked that not even a dog would eat them. A dozen new species of boletes were collected, described, and placed in the herbarium of the University of Florida. One of these, a beautiful golden species, was so abundant that a group of women picked four bushels of it in a single day for drying, canning, and pickling. *Boletus edulis* and *B. bicolor* were also very abundant. It would pay the towns of northern and central Florida to establish municipal mushroom markets with experts in charge to examine the specimens brought in before they were offered for sale. Mushrooms cannot be cultivated in Florida, but tons of delicious wild ones are going to waste in the state every year.—W. A. MURRILL.

NOTICES

The headquarters of the Mycological Society of America at the Indianapolis meetings of the A. A. A. S., Dec. 27, 1937 to Jan. 1, 1938, will be at the Washington Hotel, Washington Street, about four blocks north of the Union Station, and two blocks east of the Claypool and Lincoln which have been designated jointly as general headquarters of the A. A. A. S. The rates for the rooms, all of which are with bath, are $2.25 to $4.00 for a single room and $3.75 to $5.50 for double room.

As soon as possible after October 1st, blanks for the nomination of officers, and for application for place on the program, will be sent to members. It is hoped that these blanks will be returned properly filled at the earliest moment possible. It is also brought to your attention that by vote of the Council no person who is not a member of the Mycological Society may present a paper at the Society's meetings. It is therefore suggested that those members who have students that may wish to give papers, urge an immediate application for membership.